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SARCOSPHAERA CORONARIA

FRED J. SEAVER

(WITH COLORED ILLUSTRATION)

The species named above is one of the larger and most beautiful of the cup-fungi, and while widely distributed it must nevertheless be regarded as one of the rarer forms. The writer has never had the good fortune to encounter it in the field and, while it has frequently been sent in for determination, only once has he had the privilege of seeing the plant in a fresh condition. The name "*coronaria*" refers to the crown-like form of the open apothecia and an appropriate name would be the "Royal cup-fungus."

The colored illustrations accompanying this article were drawn by Miss Fleda Griffith from photographs sent by Miss Elizabeth E. Morse from California, where the plants were collected. The coloring was submitted to her for approval. This constitutes the first report of the species from California.

In the opinion of the writer, *Pustularia gigantea* described by Dr. Rehm of Germany from material sent from Michigan, is identical with the above. Dr. Bessie B. Kanouse, in a recent article in MYCOLOGIA (**33**: 466), regards that species as distinct from the above, and makes it *Sarcosphaera gigantea,* being distinguished by its slightly smaller spores. While there is a chance for a difference of opinion on this point, the writer still believes that the two are identical.

For a complete description and synonymy see North American Cup-fungi, page 235. The colored plate accompanying this article will be issued in a supplemented edition of North American Cup-fungi, now in the course of preparation.

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THE EFFECT OF LIGHT ON TAXONOMIC CHARACTERS IN FUSARIUM

W. C. Snyder and H. N. Hansen

(with 2 figures)

Those who are concerned with the identification and classification of *Fusaria* appreciate the influence that media, temperature, manner of seeding, and other factors in the cultural environment exert on macroscopic features of a fungus in pure culture. Especially important are these environment-induced variations when an attempt is being made to follow a system in which the species lines have been closely drawn, such as in the Wollenweber and Reinking system (**8**). Recently (**6**) a species concept has been advocated which calls for a modification of that system. In applying this concept a complete revision of the taxonomy of section *Elegans,* and later of section *Martiella* (**7**) of the genus *Fusarium,* was proposed. However, regardless of what system is followed, the successful universal use of any system is dependent upon uniform procedures and techniques in the handling of cultures. It is the purpose of this paper to treat one of the several factors which influence the growth of *Fusaria,* namely that of light, and to show the extent to which it affects the taxonomic characters of these fungi. A review of the rather extensive literature on the response of fungi to light will not be attempted here since it has been given elsewhere, in part by Coons (**1**) and by Harter (**3**).

EXPERIMENTAL PROCEDURE

Unless otherwise stated, all of the work described herein is based upon single-spore cultures obtained by the method of Hansen and Smith (**2**). All cultures used in a given experiment were made on the same day, on 2 per cent potato dextrose agar slants having the same preparation history. Five cultures of each fungus were laid horizontally in a single layer in wooden trays which were placed on tables before windows, in such a way as to be well illuminated

FIG. 1. Cultures of *Fusarium*, in triplicate, photographed after growing for 2 weeks in diurnal light (the left hand trio of each group) and in continuous darkness (the right hand trio of each group). *A–D, F. Solani* f. *Cucurbitae* from squash; *E, F. Solani* from citrus; *F, F. Solani* from sweet potato; *G, F. oxysporum* f. *niveum* from watermelon; *H, F. avenaceum* from carnation.

throughout the day but so as not to receive direct sunlight. Duplicate trays were placed directly beneath those subjected to daylight in a manner which excluded light.

Cultures used in the study included strains of the following fungi, stock cultures of which were grown in the presence of daylight: *Fusarium Solani* (Mart.) App. & Wr. emend. Sny. & Hans., imperfect form of *Hypomyces Solani* Rke. & Berth. emend. Sny. & Hans., and the form of this species pathogenic on cucurbits, namely, *F. Solani* f. *Cucurbitae* Sny. & Hans., of section *Martiella* as recently revised (**7**). Also used were *F. oxysporum* Schlect. f. *niveum* (E. F. S.) Sny. & Hans., of section *Elegans* (**6**); and *F. avenaceum* (Fries) Sacc., of section *Roseum.*

RESULTS

Characters in which marked contrasts were observed included: (*a*) pigmentation, (*b*) amount of aerial mycelium, (*c*) zonation, (*d*) amount of sporulation, (*e*) size of conidia, (*f*) septation frequency of conidia, and (*g*) perithecial formation after the fertilization of illuminated and non-illuminated cultures. These were recorded when the cultures were two weeks and again when they were four weeks old. The two readings were so nearly identical that only one set is given here, in table 1. Culture and spore characters are illustrated in figures 1 and 2.

PIGMENTATION

The colors (**5**) displayed by the fungi recorded in table 1 fell into three general groups on the basis of their relation to light. First, the flesh-ochre and cinnamon-pink pigments developed by *F. oxysporum* f. *niveum* occur only in the presence of light. Second, the ramier-blue which occurs in this same fungus seems to develop equally well in light and in darkness. The same appears to be true for the dusky green-blue of *F. Solani* from tomato, except that in this fungus the pigment is associated with conidial masses and since sporulation is greatly reduced in the absence of light, less of the pigment is observed under this condition. A third type of pigmentation is found in strains D and F of *F. Solani* f. *Cucurbitae* and *F. Solani* respectively, wherein the vetiver-green,

TABLE 1

COMPARATIVE DEVELOPMENT OF SINGLE SPORE CULTURES OF *Fusarium* WHEN GROWN FOR TWO WEEKS IN LIGHT AND DARKNESS

Key to Figs. 1 and 2	Fungus Identity	In Diurnal Light			In Darkness		
		Mycelium	Sporulation	Perithecia *	Mycelium	Sporulation	Perithecia *
A.	*F. Solani* f. *Cucurbitae* from squash	Aerial: moderate olivaceous black, trace white	Sporodochia: moderate, color of mycelium	Abundant	Aerial: moderate dark grayish olive, and white	Sporodochia: scant, color of mycelium	Trace
B.	*F. Solani* f. *Cucurbitae* from squash	Aerial: moderate white and Saccardo's umber	Sporodochia: moderate Saccardo's umber	Abundant	Aerial: moderate white	Sporodochia: scant, tawny-olive	Trace
C.	*F. Solani* f. *Cucurbitae* from squash	Aerial: moderate olive-buff, and white	Sporodochia: abundant, chamois and trace glaucous green	Moderate	Aerial: abundant, white and pale olive-buff	Sporodochia: scant glaucous green, trace chamois	None
D.	*F. Solani* f. *Cucurbitae* from squash	Aerial: moderate pale olive	Scant, no sporodochia nor pionnotes	None	Aerial: abundant citron yellow	Trace, no sporodochia nor pionnotes	None
E.	*F. Solani* from citrus	Aerial: moderate cartridge buff	Pionnotes: moderate, color of mycelium	None	Aerial: moderate, white	Pionnotes: scant, white	None

* After fertilization.
** Not illustrated in figures 1 and 2.

TABLE 1—(*Continued*)

Key to Figs. 1 and 2	Fungus Identity	In Diurnal Light			In Darkness		
		Mycelium	Sporulation	Perithecia *	Mycelium	Sporulation	Perithecia *
F.	*F. Solani* from sweet potato	Aerial: moderate white	Sporodochia: abundant chamois and asphodel green	Abundant	Aerial: abundant, white, strands and patches vetiver green and dark hyssop violet	Sporodochia: scant, only in center, chamois	Trace
**	*F. Solani* from tomato	Aerial: scant white	Sporodochia: abundant, covering slant, dusky green-blue	None	Aerial: moderate, mostly white, sectors violet-slate	Sporodochia: scant, only in center, dusky-green blue	None
G.	*F. oxysporum* f. *niveum* from watermelon	Aerial: scant white and flesh ocher. Plectenchyma: ramier blue	Pionnotes: abundant flesh-ocher	None	Aerial: moderate, white center spot pale olive-buff. Plectenchyma: ramier blue	Pionnotes: moderate, white	None
**	*F. oxysporum* f. *niveum* from watermelon	Aerial: moderate white and pale cinnamon pink. Plectenchyma: white and ramier blue	Sporodochia: abundant, salmon-buff	None	Aerial: moderate, white. Plectenchyma: white and ramier blue	Scant, no sporodochia, not colored	None
H.	*F. avenaceum* from carnation	Aerial: abundant half white, half buff pink. Plectenchyma: pompeian red	Sporodochia: moderate, salmon orange	None	Aerial: abundant Japan rose, trace white. Plectenchyma: pompeian red	Trace occasional sporodochium color of mycelium	None

dark hyssop-violet and citron yellow colors developed much more conspicuously in the absence of light.

MYCELIUM

Although no detailed observations were made on the mycelium itself, the cultures in the dark consistently showed greater mycelial growth than those in the light. Only in cases where the difference was large is this characteristic shown in table 1. The increase in aerial mycelium obtained by growing strain D of *F. Solani* f. *Cucurbitae* in the dark is shown in figure 1, *D*.

ZONATION

All cultures exposed to light developed zonation in some degree whereas none appeared in those grown in the dark. In certain strains of *F. Solani* f. *Cucurbitae*, and *F. Solani* zonation was very pronounced (FIG. 1, *C* and *F*). Some of the cultures in the dark developed sectors of differentiated growth and frequently deep folds in the mat radiated from the centers of the colonies (FIG. 1, *B*). It is recognized that light is only one factor in the environment which may cause zonation.

SPORULATION

Table 1 shows that the sporodochial strain of *F. oxysporum* f. *niveum* produced sporodochia abundantly in the light but not in the dark. In the pionnotal strain, G, of the same fungus, pionotes were produced much more abundantly in light than in the dark. In *F. Solani* and *F. Solani* f. *Cucurbitae*, which sporulate readily under most conditions of culture, the quantities produced in light and in darkness are surprisingly different (FIG. 1, *B* and *F*). The present or absence of sporodochia has been considered an important character in the taxonomy of Fusaria. In *F. avenaceum*, sporulation is plentiful along the edges of the colony in contact with the glass, when grown in light (FIG. 1, *H*), but not when grown in the dark.

The formation of perithecia and mature ascospores of *Hypomyces Solani* depended upon light as did the formation of macroconidia. Perithecial primordia appeared abundantly in the light

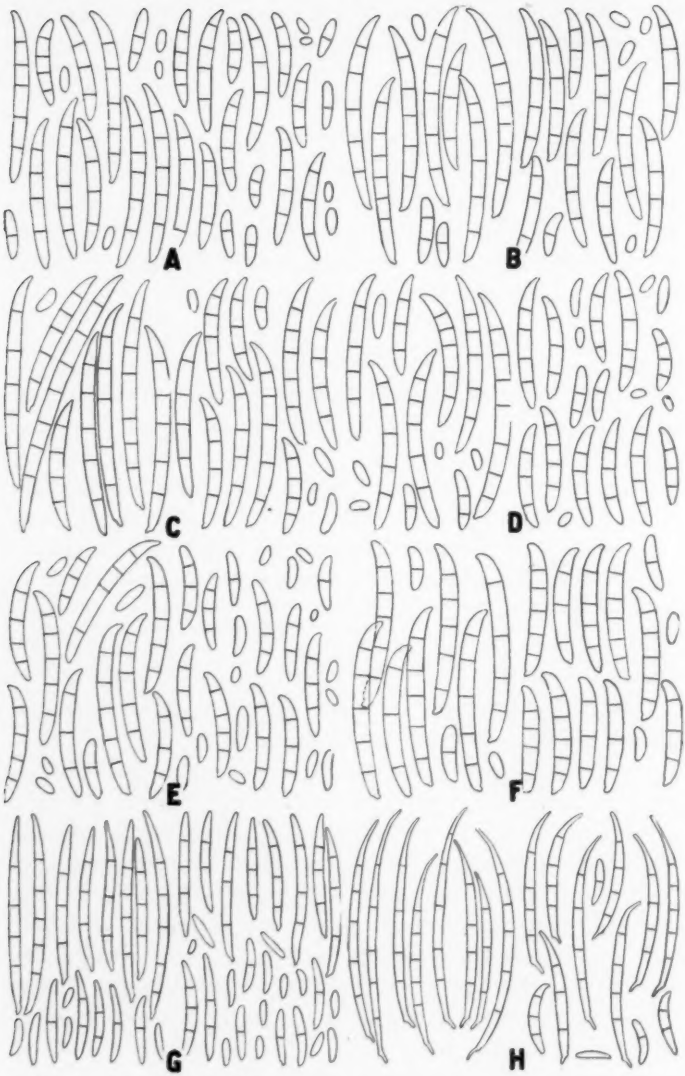

FIG. 2.

after about 2 weeks of growth, while none were apparent in cultures kept in the dark. At the end of a month both the cultures grown in light and in the dark were fertilized with conidia from the opposite sex strain. Two weeks later mature perithecia were abundant on the cultures in the light while those in the dark showed only an occasional immature perithecium or none at all. Not only was light required for the production of perithecial primordia, but it was also found that the development of these primordia into mature perithecia was favored by their continued exposure to light.

SIZE AND SEPTATION OF CONIDIA

In figure 2 is illustrated the relative size and the relative amount of septation of conidia produced on illuminated and on non-illuminated cultures. All drawings were made at the same magnification, at the same time. Samples of spores were always taken from that portion of the thallus in which the largest amount of sporulation occurred. It will be noted that those produced in light were consistently larger, and had more septa, in all cases. The relative differences in these cultures remained the same after two, four and six weeks. Since Harter's paper (3) dealt primarily with the influence of light on size and septation, no further attention need be given to them here, other than to call attention to the fact that they have been considered important in classification.

LOCALIZATION OF LIGHT EFFECT IN THE THALLUS

Two trays, each containing 40 single spore cultures of *F. Solani* from sweet potato were prepared and placed before a window, one tray covering the other. Every day at 5 P.M., 4 cultures which had grown in the light were exchanged with 4 that had been in the dark since initiation of the experiment. At the end of 10 days the surface of the cultures kept in the light the full 10 days were

FIG. 2. Camera lucida drawings made at the same magnification, of conidia produced by the *Fusarium* cultures illustrated in figure 1. Those on the left, in each case, were taken from cultures grown in light; those on the right, from cultures grown in the dark. *A–D, F. Solani* f. *Cucurbitae* from squash; *E, F. Solani* from citrus; *F, F. Solani*[*] from sweet potato; *G, F. oxysporum* f. *niveum* from watermelon; *H, F. avenaceum* from carnation.

practically covered with a sheet of macroconidia, whereas those which had remained in the dark during this time were entirely mycelial except for a small conidial mass at the point of inoculation. Forming a perfectly intergrading series were the cultures which by daily exchange had accumulated in the light tray. The longer the cultures had remained in dark before being transferred to light, the larger was the area of the mycelial part of the colony and the narrower the sporulating zone beyond it. In less than 10 days the fungus had covered the surfaces of the slants, and thereafter little or no benefit in respect to sporulation was obtained by bringing cultures from the dark into the light. Conversely, in the tray kept in darkness, the longer the period in which the fungus had been exposed to light before being placed in the dark, the larger was the area of the colony which was covered with spores and the narrower was the zone of mycelial growth which surrounded it.

These results suggest that the effect of light in stimulating conidium formation is rather localized in that part of the thallus which is growing at the time of exposure to light. The examination of growing cultures which have been brought into light after having started their development in the dark, indicates that the production of normal conidia is initiated in *F. Solani* within 24 hours after having been placed in the light, and that the zone affected is that which forms, or continues its development, after exposure to light.

DISCUSSION

The data presented in table 1. and in figures 1 and 2, show that striking temporary variations occur in the colony characters of color, zonation, amount of mycelium, amount of sporulation, and cultural topography, depending upon whether the cultures are grown in light or darkness. Furthermore, appreciable differences were obtained in the taxonomically more important characters of size, shape, septation and manner of formation of the macroconidia, between cultures of the same fungus grown in light, and in dark. These characters, especially, carry much weight in the system of Wollenweber and Reinking. The findings confirm, and supplement, those of Harter (**3**, **4**) whose work was limited to fewer fungi and fewer characters.

However, the writers' observations are in contradiction to Harter's implication that *Fusarium* cultures need be exposed to light only during the first 4 days after they are made, in order to develop similarly to those allowed to remain in light during the entire growth period. In the experiment on the localized effect of light, it is shown that the normal production of conidia, and of other light-induced effects, occur principally in those zones of growth which are initiated, or in which development is continued during exposure to light. Harter's contention that the first 4 days determine the light effect on a fungus in culture, holds true where the smear method of seeding slants, which he used is employed. The explanation of this difference between single spore and smear cultures would seem to lie in the fact that in smear cultures a multitude of local foci of growth over the entire slant causes mycelial development to be practically completed in 4 days, whereas in the single spore seeding the thallus develops continuously from a single focus for about 10 days before it covers the same area of slant surface and completes its mycelial development. The same difference may occur in a slightly smaller degree between smear cultures and those prepared in the usual manner of transferring a small mass of fungus hyphae with or without its supporting medium.

The single spore method used here has an advantage over other transfer methods in that it permits a more detailed analysis of the differences induced by the modification of an element in the environment. It reveals such characters as zonation and pigmentation in a way impossible with the smear method. The differences between the ratios of 3-, 4-, and 5-septate spores that Harter (**4**) found in individual tubes perhaps may also be traceable to his method of seeding. Certainly it may not become immediately apparent by such a method when variants appear in the stock culture. In the work reported here, no differences in septation ratios or in any other spore character were found between the individual single spore cultures of a stabilized homotypic clone when grown in the same environment.

The importance of light in the inception of perithecial primordia and in the development of perithecia following fertilization is

shown to be marked in *Hypomyces*. The implications of this finding, in respect to the development and location of perithecia of such fungi in nature and in the laboratory, are apparent.

It becomes clear that it is essential where *Fusaria*, or other similar fungi are concerned that the cultures be allowed to develop in the presence of light, if the maximum development of colony and of morphologic characters employed in taxonomy is desired; and that the same considerations apply to the perfect stages of these and probably of many other fungi.

SUMMARY

Marked differences in the characters ordinarily used in taxonomy are revealed when 10 strains of 3 species (*F. Solani, F. oxysporum* and *F. avenaceum*), representing 3 sections of the genus *Fusarium*, are grown in light, and in darkness.

The quality of the colony and morphologic characters were affected, as were also the quantity and to a certain degree the occurrence of such characters as perithecial primordia.

Evidence is given which indicates that the effect of light is produced on that portion of the thallus which is actively growing at the time of exposure. Single spore cultures subjected to light only for the first 4 days of growth, fail to develop in the same manner as those allowed to remain in light.

It is concluded that such characters as color, zonation, type of colony, presence or absence of sporodochia; size, shape and septation of macroconidia; and even the occurrence of a perithecial stage, can not be employed successfully in taxonomy unless these fungi are grown in adequate light.

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COMPARATIVE STUDIES ON THE PRIMARY AND SECONDARY ZOÖSPORES OF THE SAPROLEGNIACEAE. I. INFLUENCE OF TEMPERATURE [1]

S. B. SALVIN [2]

(WITH 2 FIGURES)

INTRODUCTION

Optimum and other conditions for zoöspore production have been investigated by Cotner (**1**), Jones and Drechsler (**5**), de Bary (**3**), and others. No one, however, has published a comparative analysis of the physiological properties of primary versus secondary zoöspores, using the rate of movement as an index. Such properties would include the reactions of the swarmspores to various temperatures, viscosities, hydrogen-ion concentrations, salinities, and degrees of aerobiosis. The present study deals chiefly with one of these properties—namely, the effect of temperature on the zoöspore activity. In a later publication, the author hopes to describe the influence of the other conditions on both primary and secondary zoöspore activity.

MATERIALS AND METHODS

Five members of the Saprolegniaceae were used in the following work: *Saprolegnia delica* Coker, isolated from Houghton's Pond in the Blue Hills Reservation of eastern Massachusetts; *Saprolegnia* W, a non-sexual form obtained from sand at the edge of Walden Pond, Concord, Mass.; *Achlya flagellata* Coker, collected from Beaver Brook, Waverly, Mass.; *Thraustotheca clavata* (de Bary) Humphrey, isolated from sludge from Ithaca, N. Y.; and

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, no. *190*.

[2] The writer expresses his gratitude to Prof. W. H. Weston, under whose guidance this investigation was conducted, for his valuable suggestions and continued interest.

Dictyuchus 2, another non-sexual form, obtained from an infected *Avena* seed submerged in a hay infusion medium for protozoa.

All cultures used were grown from single-spore inoculations, and all experiments carried out in sterile, redistilled, well-aerated water. The sporangia and zoöspores were taken from young, vigorous mycelia, 24 to 48 hours old, which had been grown at temperatures approximating 25° C. At the time of the experiment, a mature sporangium, ready to discharge its spores, was cut free from the mycelial mass and carefully transferred, with a small portion of the attached hypha, to a hanging-drop which had already attained the required temperature.

The experiments were conducted in rooms the temperatures of which were 5°–10° C. below those of the micro-stage constant temperature chambers (manufactured by the Chicago Chemical and Surgical Company), in which the zoöspore preparations were enclosed.

After the hanging-drop slide with the sporangium enclosed had been inserted in the micro-stage, a half-hour was allowed to elapse before any readings were taken in order to make certain that equilibrium had been attained within the apparatus. After this interval and after the emergence of the zoöspores, the time required for the swarmspore to swim across the field of the microscope was determined by means of a stop-watch. Only those times were recorded in which the zoöspore moved in a reasonably straight line and through a diameter of the field. If the spore spiraled or twisted excessively or if it swam along a chord of the field, the reading was rejected. In this way, fairly consistent results were obtained. For example, when three sets of readings were chosen at random, the close agreement of the figures was obvious, as substantiated by the values of the mean, median, mode, and standard deviation (TABLE 1).

The actual rate of movement of the zoöspores was obtained by dividing the diameter of the microscope field (0.33 mm.) by the time required for the spore to traverse this distance.

RESULTS

For understanding the activity of the zoöspores, it is well to review briefly the structure and method of swimming of the two

TABLE 1

TO SHOW AGREEMENT IN THE RATES OF ZOÖSPORE MOVEMENT IN 60 READINGS OF EACH OF THREE SETS OF DATA

Genus and Condition	Range	Mean	Median	Mode	Standard Deviation
Dictyuchus 2 at 15° C. 60 readings.....	2.5–2.9 sec.	2.69 sec.	2.7 sec.	2.7 sec.	0.0855
Saprolegnia W at 10° C. 60 readings.....	2.2–3.0 sec.	2.56 sec.	2.5 sec.	2.5 sec.	0.672
Dictyuchus 2 at 30° C. 60 readings.....	1.2–1.5 sec.	1.33 sec.	1.3 sec.	1.3 sec.	0.086

types. The primary zoöspore of *Saprolegnia* normally emerges through the papilla of the sporangium as a motile, pip-shaped entity with two anteriorly attached flagella, each of the same length and slightly longer than the body of the zoöspore proper. Each of these two flagella is inserted in a highly staining granule, situated at the apical end of the anteriorly located nucleus. The cytoplasm of the swarmspore is differentiated into two parts: a central, dense mass, enclosing most of the nucleus; and a vacuolar, less dense peripheral zone (1).

The secondary zoöspore of *Achlya, Saprolegnia, Dictyuchus,* or *Thraustotheca* emerges from its cyst as a subovoid body with two rather long flagella—both inserted laterally, with one extending anteriorly and the other posteriorly. By means of a basal granule, each of the flagella is associated with the nucleus, which is centrally located and surrounded by a rather dense mass of cytoplasm. The rest of the zoöspore body consists of vacuolar, less dense cytoplasm. In its swimming movements, the primary zoöspore was observed traveling in an irregular spiral course, with a rotation usually in a counter-clockwise direction—in contrast to the secondary in which the rotation was generally clockwise.

The primary zoöspores, discharged from the sporangia of *Saprolegnia* W and *S. delica,* differed from the secondary, obtained from *Achlya flagellata, Thraustotheca clavata, Dictyuchus* 2, and *Saprolegnia* W, in such characters as the rate of movement, the length of the swarming period, and the reaction to various temperatures. When the two types of zoöspores were subjected to a temperature of 25° C. (77° F.), the secondary zoöspore progressed at a more

rapid rate, with the primary averaging 110 μ per second, and the secondary of *Saprolegnia, Achlya,* and *Dictyuchus,* 236, 195, and 201 μ per second respectively.

In the length of the swarming period, the two types of zoöspores differed, with the primary swarming on an average from 12 to 35 minutes and the secondary from 25 to about 450 minutes. In one instance, the total swarming period of the zoöspores of the non-sexual *Dictyuchus* was strikingly long, having been observed for over eight hours, at the end of which time the writer had to discontinue the observations; yet, almost all of the zoöspores were still swarming and there were no empty cysts in the hanging-drop to indicate that any repeated emergence had begun.

The primary and secondary zoöspore rates were studied and compared over a range from 10° C. (50° F.) to 30° C. (86° F.) (FIG. 1). The secondary typically carried on normal activity over wider extremes of temperature; swarmed at a faster rate and for

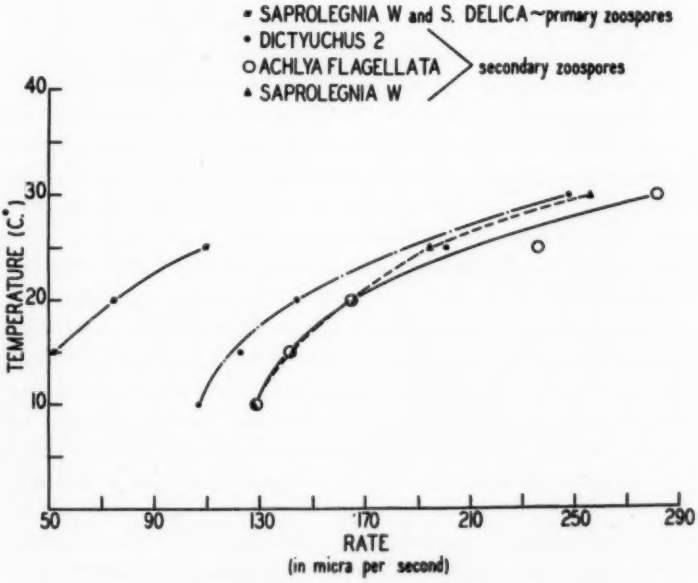

FIG. 1. Graph, illustrating the influence of temperature on the movements of primary and secondary zoöspores.

a longer period; almost trebled its speed over the range of normal activity; had its slowest rate equaling the fastest for the primary; was less inclined to gyrations; had its speed range from 170 μ per second at 10° C. to 282 μ per second at 30° C.; possessed an average "temperature coefficient" (Q_{10}) of 1.36 to 1.66 at the temperatures 15° to 25° C.; and had a Q_{10} extend in *Saprolegnia* from $1.28_{(10°-20°)}$ to $1.71_{(20°-30°)}$, in *Achlya* from $1.30_{(10°-20°)}$ to $1.58_{(20°-30°)}$, and in *Dictyuchus* from $1.36_{(10°-20°)}$ to $1.71_{(20°-30°)}$. The primary, on the contrary, displayed normal activity over a narrower temperature range; swarmed at a slower speed and for a shorter time; hardly doubled its rate over the range of normal activity; carried on more gyrate movements; had a speed extending from 57 μ per second at 15° C. to 110 μ per second at 25° C.; and was characterized by a Q_{10} of 1.93 at the temperatures of 15° C. to 25° C.

In addition to the general conclusions mentioned, certain specific observations at the extremes of the temperature range are of interest. At 10° C., the primary zoöspores of *Saprolegnia* did not behave in a normal manner after their emergence from the sporangium. In some instances, when they encysted almost immediately after emerging, they all formed a loose mass of encysted spores near the mouth of the sporangium. In others, some of the primary zoöspores encysted almost at once, and the rest whirled about in very narrow spirals; whereas in still others, some of the zoöspores wiggled within a quite limited area and the others rotated their anterior ends slowly while the posterior remained stationary.

At 30° C., primary zoöspore activity was also abnormal. Instead of swimming in a relatively straight line, the zoöspores circled and spiraled, twisted and turned, and carried on vigorous vibratory movements. In addition, on two separate occasions, fusion of zoöspores was observed—a phenomenon never previously recorded in the literature. As two zoöspores circled near each other, their anterior portions accidently came into contact and then became attached apparently quite firmly. Although it was quite difficult to observe exactly what happened during the subsequent two to three minutes due to the intense and irregular whirling motion of the zoöspores, it was noted that the two bodies slowly fused, after

which activity suddenly ceased. About a half-hour later, the resulting cyst gave rise to a short hypha of germination.

When attempts were made to study the movement of secondary zoöspores of *Saprolegnia* W and *Dictyuchus* 2 at 35° C. (95° F.), no results were obtained for two main reasons: first, the encysted spores, whether within or without the sporangium, mostly failed to give rise to motile entities, but germinated *in situ* by giving rise to a short hypha; secondly, in the very few cases where secondary zoöspores were emitted, the path of motion was so irregular as to make the recording of accurate results impossible.

DISCUSSION

It is known that many vital processes in both plants and animals are accelerated by a rise in temperature. This relation between the velocity of a reaction and the temperature may be expressed by the ratio,

$$\frac{K_{t^\circ}}{K_{t^\circ-10}},$$

in which "K" indicates the frequency of a process at each of two temperatures 10° C. apart. This ratio, known as the "temperature coefficient," or Q_{10}, has a value of from 1.2 to 1.5 for certain physical processes, such as vaporization, and from 2 to 3 for many chemical reactions.

However, the use of the "temperature coefficient" does not permit analysis of frequent alterations of rate with change in temperature. Hence, there has arisen the use of the "temperature characteristic," μ, as applied to the Arrhenius equation:

$$\frac{K_2}{K_1} = e^{\frac{\mu}{R}\left(\frac{1}{T_1}-\frac{1}{T_2}\right)},$$

where K_1 and K_2 are the velocity constants at temperatures T_1 and T_2, e is the base of the natural or Naperian logarithm (2.718), R is the gas constant, and μ is the "thermal increment" or "temperature characteristic" (**2**).

Although it has originally been suggested that the "temperature characteristic" depended on the formation of active molecules of a reaction, the investigations of Rice (**6**) gave adequate basis for

the belief that the controlling factor was the rate of activation of the catalyst in the process. Thus, for those reactions known to be catalyzed by the hydrogen ion, μ has a value of 20,000 calories; by iron, 16,200; by the hydroxyl ion, 11,500; and by copper, 8,000.

The velocity of a vital reaction is assumed to be controlled by the velocities of closely associated systems of chemical reactions, with the slowest portion of the chain determining the speed of the process as a whole. As Crozier (2) has so aptly stated, "the virtual velocities of two catenary reactions

$$A \underset{k_1}{\rightarrow} B \underset{k_2}{\rightarrow} C$$

with velocity coefficients k_1 and k_2, and having different temperature characteristics, might be so related that at a certain temperature these actual velocities are dynamically identical, while below that temperature $A \rightarrow B$ would be the 'slow reaction,' and above that temperature $B \rightarrow C$."

In the analysis of the data concerning the swimming movements of the primary and secondary zoöspores of the Saprolegniaceae, it is assumed that the forward progress depends on a chain of chemical reactions, and that the same amount of energy is expended in the traversal of a given distance. Hence, in its relations to temperature, the swimming movements of the zoöspores should reflect the properties typical of the controlling process.

The "temperature characteristics" (μ) for the primary zoöspores and for the secondary zoöspores were found to be 10,450 and 8,100 respectively (FIG. 2), remaining constant throughout at the temperatures from 15° to 25° C., and from 10° to 30° C. respectively. In addition, the secondary zoöspore with its faster rate of movement probably has a higher rate of oxygen intake than the primary, since Gray (4) showed that in *Mytilus* the rate of ciliary movement was proportional to the oxygen consumption.

At this point, an attempt might be made to identify the reactions the rates of acceleration of which are characterized by μ values of 10,450 and 8,100. The magnitude of the former value would indicate that the controlling reaction might fall within the class of processes catalyzed by the hydroxyl ion, whereas that of the latter would suggest that the reaction was under the control

of a copper catalyst. The foregoing values also suggest, first, that the dominant and limiting process in the secondary zoöspore is identical in all the genera studied, and secondly, that even within a single species, as soon as there is a transition of the zoöspore from a primary to a secondary type, not only is there a change in the general morphology, but in reality a transformation in the fundamental biochemical constitution.

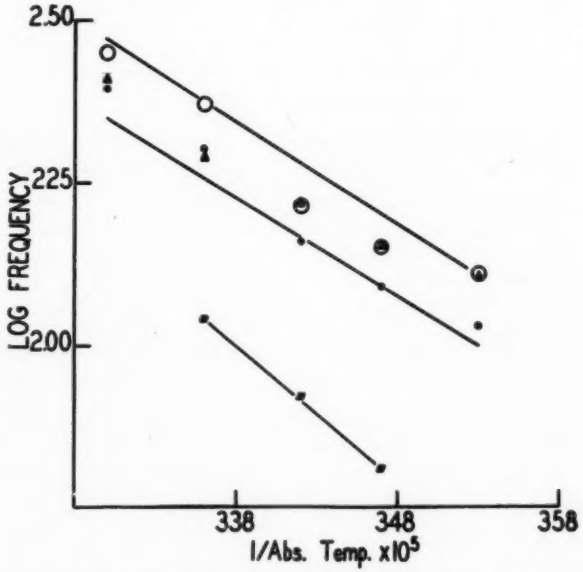

FIG. 2. Graph, illustrating the temperature characteristics for the frequency of zoöspore movements. (Legend is the same as in Fig. 1.)

From the previously described experiments, it becomes evident that the secondary zoöspores are by far the more efficient in the survival and distribution of the fungus because they are more resistant to temperature changes and extremes; and swarm more readily, at a faster rate, and over a greater period of time. Furthermore, they are capable of carrying on repeated emergence (7).

SUMMARY

The activity of the primary and secondary zoöspores in four genera of the Saprolegniaceae was studied and compared at tem-

peratures from 5° to 35° C. Under carefully controlled conditions, primary zoöspores, for example, swarmed for a shorter period, carried on normal activity over a narrower temperature range, moved at a slower rate, and had a higher temperature coefficient than the secondary. Since the two types have different "temperature characteristics," their activities are probably controlled by different catalysts.

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STAGES IN THE DEVELOPMENT OF THELOTREMA INTERPOSITUM

G. T. JOHNSON [1] AND W. L. BROWN [2]

(WITH 9 FIGURES)

INTRODUCTION

Thelotrema is the type genus of a family of discomycetous lichens which, though widely distributed throughout the world, occurs primarily in tropical and subtropical regions. With the exception of a few remarks by Redinger (**1936**), based upon work with herbarium material, no developmental study of the genus has appeared to date. Material of *Thelotrema interpositum* (Nyl.) Müll. Arg. was obtained near Perkinston, Stone County, Mississippi, in December, 1938, June, 1939, and August, 1940 (killed and fixed in formol-acetic-alcohol and in Navashin's solution). Permanent slides, prepared after embedding this in low-viscosity nitrocellulose by the method described by Koneff and Lyons (**1937**), were supplemented by smears prepared in the field. Examination of this material has enabled the writers to ascertain stages in the development of the species which seem to be of sufficient interest to justify their description and illustration at this time.

STAGES IN DEVELOPMENT

Crustose lichens usually have very simple thalline structure. Those like *Thelotrema,* which occur primarily on the bark of living trees, are often more or less immersed in the substrate. Hyphae of *T. interpositum* penetrate several cell-layers into the bark, but the evident portion of the thallus is superficial (FIG. 3). The superficial tissue is arranged in a pustule-like manner, in which the algae are located near the outside, forming a layer above some small air cavities and surrounded by the fungal component. Apothecial

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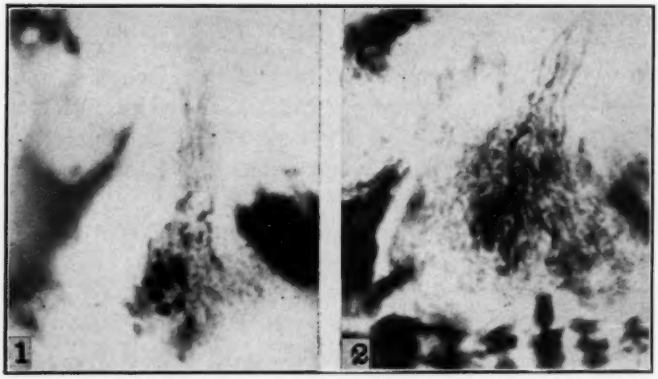
initials arise below the gonidial layer, just above the uppermost layer of bark cells. Primordia have been found in all of our collections and they may apparently be present at all periods of the year.

The primordial coil of hyphae expands and ascogonia are developed. Throughout this and subsequent processes, the initial gradually becomes embedded in and somewhat surrounded by bark cells (FIGS. 2 and 3). All apothecia arise from initials containing



FIGS. 1 and 2. Apothecial initials destained sufficiently to show the ascogonial coils. × approx. 440.

several ascogonia (FIGS. 1–5) and each ascogonium is composed of a coil of large, dark-staining, uninucleate cells (FIGS. 1 and 2). A trichogyne, an elongate series of uninucleate cells, is formed which extends from the coil to a considerable distance above the thallus (FIGS. 4 and 5). The trichogyne may vary from 60 to 112 μ in length and project as much as 39 μ above the surface of the bark.

Examination of the slides prepared by smear technique revealed that some of the apothecia were of more than usual interest. The apothecia in question were evidently younger than others, judged by size and shape, but there were no other evident morphological differences. In many of these neither asci nor spores could be found, but numerous small bodies, similar in appearance to certain types of spermatia were observed (FIGS. 6–9). These were borne on the structures that in older apothecia would unhesitatingly have

been called paraphyses. Each "paraphysis" was filamentous and composed of uninucleate cells. The spermatium-like bodies developed on the "paraphyses" in the manner shown by figure 7. They were borne laterally on the filaments, and though several were usu-

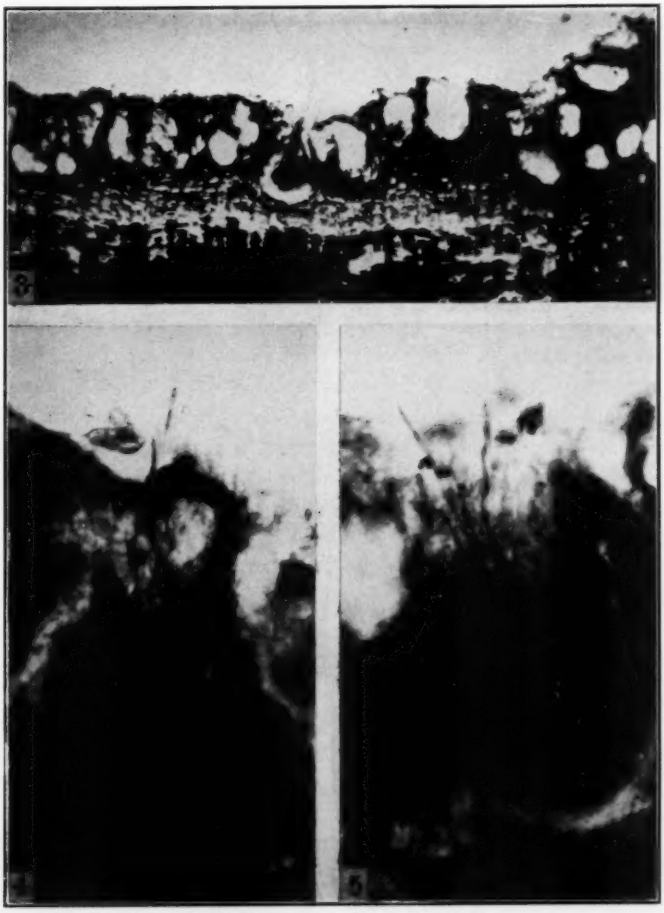


FIG. 3. A section of the thallus illustrating the location of the apothecial initial in relation to the substrate. × approx. 100. FIGS. 4 and 5. Apothecial initials stained so as to show the trichogynes projecting above the surface of the thallus. × approx. 440.

ally found on a single filament (FIGS. 7 and 9), we never observed more than one attached to a single cell. When the bodies were mature they contained only one nucleus and the cells upon which they were borne were also uninucleate. They were hyaline, 3 to 7 μ in length, and fusiform to ovoid or bacilliform. We feel certain that they were produced within apothecia in all cases, because we have observed them in apothecia within which mature spores were also borne. The "paraphyses" could easily be traced into the layer of ascogenous hyphae, but it was not possible to determine if the two were connected.

Few stages intermediate between primordia provided with trichogynes and mature apothecia have been found. The spermatium-like bodies have been seen in the neighborhood of trichogynes, but not in actual connection with them. Whether or not spermatia fuse with trichogynes, the latter soon degenerate and a complex system of ascogenous hyphae is formed. Asci apparently arise from this through typical crozíers. The young ascus is uninucleate from an early stage.

The asci of *T. interpositum* are greatly elongated and devoid of a conspicuous sheathing membrane in their younger stages. In many other lichens the asci are more clavate, and the sheathing membrane is more distinctly visible, even in young asci, particularly in the nature of a thickened tip.

Dodge (**1928**) has described spore formation in asci with fewer than eight spores. In these cases the definitive nucleus divides three times. Eight nuclei result, one of which functions in the formation of the spore while the rest degenerate. *T. interpositum* is interesting both because the ascus is one-spored and because the spore is muriform. Division of the fusion nucleus takes place and eight nuclei are probably formed. A thick membrane is secreted around one of these. The membrane is very distinct, but it surrounds only a small portion of the epiplasm of the ascus. Two or more spore initials may be found, but asci containing these probably do not develop since only one mature spore per ascus has been observed. A great deal of deeply staining chromatin-like material is present in the asci. This may represent decomposed nuclei, but further evidence is required, since even young asci contain a substance which stains in a similar manner. The young spore and

its membrane increase in size until the interior of the ascus is well-filled.

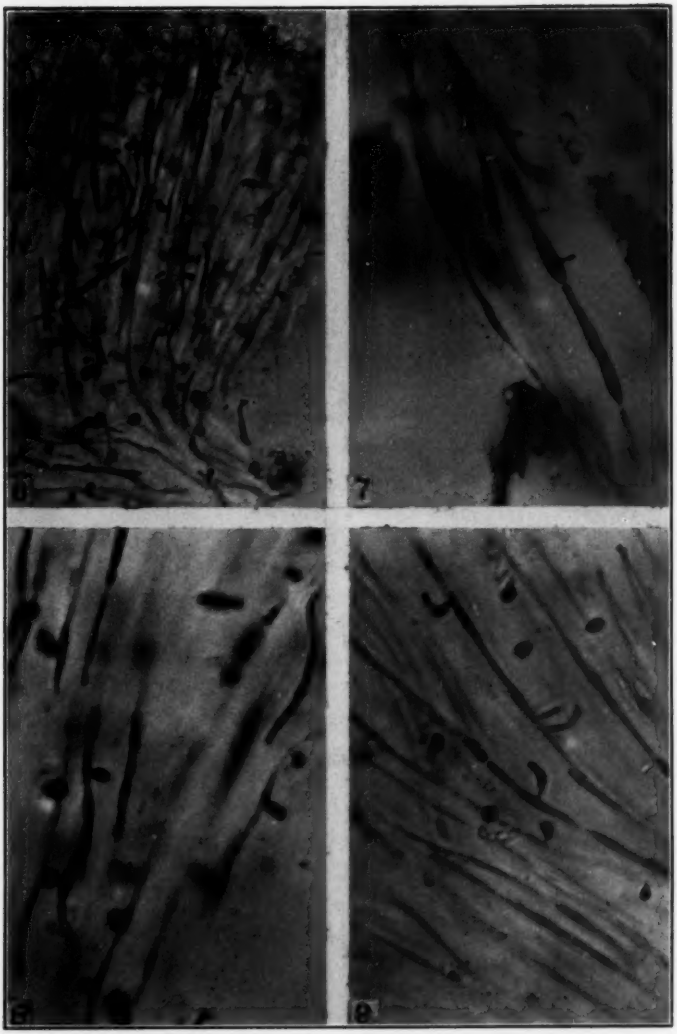
DISCUSSION

The discovery of spermatium-like bodies attached to "paraphyses" is the most important contribution reported in this paper. To the writers' knowledge, no structures exactly like these have previously been described for any lichen. Technical difficulties are encountered in the study of lichen life-histories, and far too little is known about developmental stages, even in connection with the species that have been studied in the greatest possible detail. The use of smear techniques (Johnson and Brown, **1941**) resulted in the discovery of these unusual structures in *Thelotrema,* and we have found such methods the most convenient for their study. The bodies are probably asexual spores or elements which have some function in a sexual process.

Asexual spores are rare among lichens. Those reported by Bornet (**1873**) for *Arnoldia* (*Physma*) *minutula* are quite different from the structures we have described (Miss Smith, **1921**, noted that Bornet might have had a parasitic imperfect fungus). In *Caloplaca aurantia* var. *callopisma* Steiner (**1901**) described conidia similar to the structures in *T. interpositum* in that the conidia were borne within apothecia and even within those containing asci and spores. In *T. interpositum,* however, several bodies are produced laterally rather than a single one terminally, and the apothecia in which they are borne are normal rather than abnormal in appearance. In *C. aurantia* var. *callopisma* the conidiophore increased in length during the production of the conidium and the spore was finally borne above the disk when it was mature. In contrast, *T. interpositum* produces its bodies within the apothecium (at maturity they are found free within it), the apothecium is not open and disk-like as in *Caloplaca,* and no mechanism for the dissemination of an asexual spore is evident.

One should bear in mind that the differences between conidia and spermatia sometimes seem very slight, as work on *Neurospora* (Dodge, **1932**; Backus, **1939**) has clearly shown.

The strongest evidence yet available that spermatia in other lichens function in a sexual process comes from observations re-



FIGS. 6–9. From acetocarmine smear preparations. FIG. 6, spermatium-like bodies attached to 'paraphyses,' × approx. 440; 7, spermatium-like bodies in young stage of development; 8 and 9, spermatium-like bodies near maturity. FIGS. 7–9, × approx. 900.

porting the close attachment of spermatia to trichogynes. We have found bodies similar to those borne in the young apothecia near trichogynes of *Thelotrema,* but have never seen the two in organic union. There is some evidence that these spermatium-like bodies are similar to spermatia in function, but further data is necessary to determine the validity of the supposition.

A single case is known, however, which adds considerable weight to such an opinion. In *Collemodes Bachmanianum* (Bachmann, **1912**, **1913**; Fink, **1918**) spermatia are not found enclosed in spermagonia, but both terminal and lateral spermatial groups are borne on spermatiophores embedded within the thallus. The characteristics of these undoubted spermatia are sufficiently similar to those of the spermatium-like bodies of *T. interpositum* to indicate that the structures may be homologous.

It has been suggested that copulation may occur between these elements, but neither this reaction nor any evidence to support the suggestion has been observed. The writers are aware that paraphyses are reported to cut off conidium-like formations from their tips (*e.g.,* Schmidt, **1939**) and that ladder-like cross-connections are sometimes formed between paraphyses, but there seems no similarity between either of these and the bodies we are inclined to consider spermatia. Regardless of the interpretation now placed on these structures, however, their discovery is somewhat startling, and it has resulted in sufficient evidence to indicate that the description of the complete life-history of this lichen will be of considerable interest to morphologists.

SUMMARY

1. The apothecia of *Thelotrema interpositum* arise from a group of ascogonial coils, each of which is provided with a trichogyne. This initial is similar to that which has been described in many lichen groups.

2. Spermatium-like bodies have been found attached to the "paraphyses" of some of the apothecia. These are unusual in their nature and place of occurrence, but they seem best interpreted either as asexual spores or (more probably) as spermatia. There is some evidence that they may fuse with the trichogyne.

3. Asci are formed from ascogenous hyphae, apparently through typical croziers. The asci first grow in length and the sheathing membrane may not appear until a later stage. After division of the definitive nucleus, one of the resulting nuclei is delimited in a small unit of the epiplasm by a very distinct membrane and this spore initial grows to fill the ascus. Most asci contain some puzzling chromatin-like material.

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PODAXIS PISTILLARIS. II

ELIZABETH EATON MORSE

A former paper on *Podaxis*[1] by the writer concluded with the hope that the relationships of this genus would yet be fully worked out. This hope is in course of realization. Very young material from the Colorado Desert was supplied by E. V. C. Northrop, El Centro, Calif., to both Fischer and Brasfield who agreed that very early stages of *Podaxis* and *Secotium*[2] are similar. Brasfield found that in *Secotium* the hymenium consists of closely compacted basidia which cover the surface of crumpled gills, whereas the writer found in *Podaxis* that the gleba develops into capillitium, basidia, and variable spores, with no suggestion of lamellae; furthermore, *Secotium* has no capillitium.

The classifications of *Podaxis* and *Secotium* made by Brasfield and Fisher are quite different; the former places them together in Secotiaceae in Hymenogastrales. In Die Natürlichen Pflanzenfamilien, Vol. 7a, 1933, Fischer makes a separate "Unterreihe" for *Podaxis*—Podaxineae—to include two families in equal rank, Podaxaceae and Secotiaceae—*Podaxis* being the sole representative in its family. The writer favors Fischer's disposition of this genus.

The powdery gleba of *Podaxis* at maturity points to relationship with Lycoperdaceae proposed by Lloyd and accepted by Clements and Shear.

The phylogenetic position of *Podaxis* still remains in doubt, as in the time of de Bary (1887), although some progress has been made towards its determination.

There are other stalked gasteromycetes produced in sandy regions, namely, *Phellorinia* (which also has fascicled basidia like *Podaxis*), *Gyrophragmium*, *Montagnites*, *Batarrea*, *Tulostoma*, *Calostoma* and *Dictyocephalos*, whose respective relationships to *Podaxis* and to one another are not yet determined.

Specimens of *Podaxis* collected by W. H. Long from alkaline or salty soil in Brownsville, Texas, Nov. 1916, appeared to be identi-

[1] Mycologia **25**: 1–33, 1933.

[2] Univ. of Iowa Studies **17**: 200–206, 1937.

cal with those collected in other regions in the southwest except that the spores are uniformly smaller, averaging only 3–7 × 3–5.25 μ instead of 8.5–19 × 8.5–15 μ (Long Herbarium, Albuquerque, N. M., *6884*). Carleton Rea studied the material from Doctor Long and reported that "it is a small-spored race of *Podaxis*." Heim, in "La formation des spores chez les *Podaxon*" (1932), states that spores may exhibit great variation in form and size, probably correlated with nutritional or other environmental factors. Possibly we should be prepared to accept even marked variation in spore size according to favorable or unfavorable conditions of moisture, temperature, soil or salinity; however, the writer would designate the above mentioned Texas material as a variation of *Podaxis pistillaris* (L. ex Pers.) Fries **paurospora** var. nov. Dearn., a name suggested by Dr. Dearness.

We are grateful to J. B. Cleland for specimens collected in Australia which he called *P. loandensis*. These have been examined by Lee Bonar, V. M. Miller, and the writer without finding any specific differences from our *Podaxis pistillaris*; so far it still appears to the writer that probably there is only one variable species of *Podaxis*.

Podaxis pistillaris was found near Gardner Bay, Hood Island, Galapagos, "on the equator," by Stewart, June 26, 1906, communicated by Alice Eastwood and John Howell, Academy of Sciences, San Francisco, No. *7532*, recorded by Bonar in Proc. Calif. Acad. Sci., July 20, 1939.

The northern range of *Podaxis*, 40° N. Lat., was extended by about 100 miles when S. M. Zeller discovered a specimen in a strawberry patch at Bend, Oregon, June, 1935.

The species is widely distributed in California, Arizona, Nevada, New Mexico and Texas—we have had many new collectors and additional localities represented since 1933. Furthermore, there are records which show that *Podaxis* has been collected from the Sinaitic peninsula in Asia, from termite mounds in Madagascar and South Africa, in fact from every continent.

Further reports are solicited, especially of the new small-spored variety, *paurospora*.

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CORDYCEPS STYLOPHORA AND CORDYCEPS RAVENELII[1]

E. B. MAINS

(WITH 2 FIGURES)

Cordyceps stylophora was collected by Ravenel in South Carolina and was described and illustrated by Berkeley in 1857 (**1**). Ravenel distributed it in his Fungi Car. V: 49. It has been rarely collected. It was next obtained by G. H. Hicks in April 1892 near the Michigan Agricultural College at East Lansing, Michigan. A part of this collection is in the Herbarium of the University of Michigan and bears a notation that it was determined by Ellis. Longyear (**3**) reported it in 1904. Roland Thaxter collected a specimen in August 1896 at Burbank, Tennessee (**4**). Petch (**7**) has reported a collection (Cornell *14808*) by H. H. Whetzel from Cayuga Lake Basin, New York, made in November 1902. L. E. Wehmeyer obtained two specimens at Brookside, Nova Scotia, on July 25, 1931. A. H. Smith made a number of collections at Warrenburg and Catlin Lake, New York, during August and September 1934 and from Oakland County, Michigan, in August and September, 1937 and October 1938.

Most of the collections were immature. In the original description of the species Berkeley states that he had not seen "ripe asci." Massee (**6**) described the asci and spores. The latter are given as filiform, slightly curved when free, multiseptate, 125–135 $\times$ 1 μ, the component cells 3.5 μ long. He cites only the type specimen, Ravenel *1325*. Petch (**7**), however, states that he examined the type specimen and it was "quite immature." The Cornell specimen was also found to be immature. Mains (**5**) has also noted that most of the collections from Tennessee, New York and Michigan were immature. The Hicks' specimen was the nearest to full maturity, the asci being well developed and the ascospores

[1] Paper from the Department of Botany and the Herbarium of the University of Michigan.

differentiated. It had not reached full maturity since only a few free spores were found in a mount and there is no evidence of spore discharge from the ostioles of the perithecia.

In 1938, an attempt was made to obtain thoroughly mature clavae when A. H. Smith discovered on July 10 a number of immature clavae on a rotten log near Milford, Michigan. These were inspected from time to time during the summer for exudation of spores. This did not occur and on October 24 they were collected. The clavae were in good condition. The perithecia contained asci but the spores were not fully developed. The persistence of clavae for more than three months without reaching full maturity was puzzling.

On May 29, 1939, the log at Milford was again visited and a single clava was found showing abundant exudation of spores from the ostioles of the perithecia (FIG. 1, *A*, and *B*). Of the various collections, those obtained in April and May were the most mature while those later in the season, July to October well all much less mature. This suggested the possibility that the clavae might overwinter and mature in the spring. Consequently in September 1939 the locality at Milford was again visited and eight clavae were found. Five of these had developed perithecia as shown by the presence of ostioles. These were marked by driving nails in the log beside each. In late April of 1940, the log was again visited and four clavae were found to have survived the winter, two with perithecia and two sterile. The fertile clavae were found to have well developed asci and differentiated ascospores which issued from some of the asci when mounted on a slide under a cover glass. They were not completely mature since spores were not exuding from the ostioles of the perithecia. The clavae of this species therefore can overwinter and mature in the spring. These observations strongly suggest that they start their development about July, the perithecia developing during the summer and the asci in late summer and autumn. They then overwinter and the ascospores reach maturity by the middle or the last of May.

It is now possible to give a more detailed description of the species as follows:

Clavae, single or occasionally two, ochraceous-tawny to dark cinnamon-brown, 1.5–4.5 cm. long, the fertile portion a cylindrical

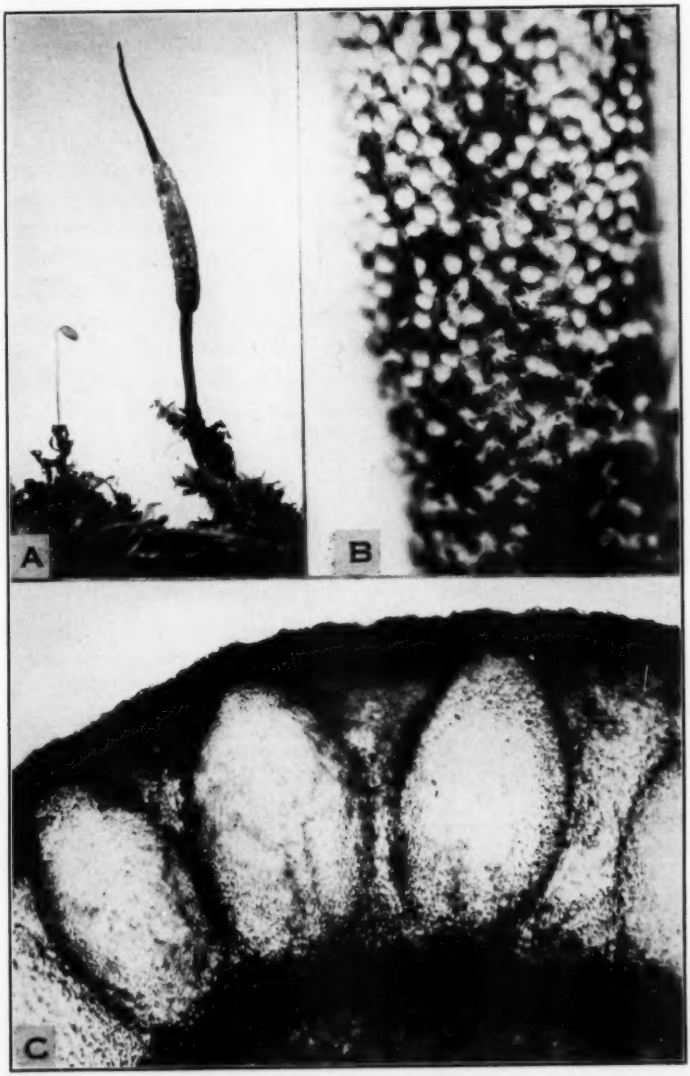

FIG. 1. *Cordyceps stylophora.*

swelling in the middle third of the clava, about 2 mm. thick, narrowed below into a sterile stipe about 0.8 mm. thick, attenuated above into a long acuminate sterile apex, the fertile portion smooth when fresh, longitudinally furowed when dry, punctate with the dark ostioles of the perithecia, the stipes surrounded at their bases by a brown mycelium; perithecia entirely embedded in the stroma, narrowly flask-shaped or ovoid, 240–420 × 144–240 μ; asci clavate-cylindric, somewhat attenuated below, slightly narrowed above, 170–220 × 8–10 μ; ascospores fusoid-cylindric, slightly narrowing at the ends, 102–164 × 2–3 μ, overlapping in the ascus, multiseptate, the cells 12–20 μ long, not regularly breaking into segments.

From coleopterous larvae in rotting logs, South Carolina, Tennessee, Michigan, New York and Nova Scotia.

Sections through the fertile portion of a clava (FIG. 1, *C*) show a somewhat similar differentiation as that described for *Cordyceps agariciformia* (*C. capitata*) by Jenkins. The differentiation is more pronounced and can be easily demonstrated from unstained, free-hand sections. There is a central cylinder composed of nearly colorless, longitudinal, parallel hyphae. Surrounding this is a thin intermediate layer of brownish, compactly interwoven hyphae. Beyond this is the perithecial layer. The perithecia are seated on and appear to arise from the intermediate layer. In the perithecial layer the perithecia are surrounded by hyphae which are nearly colorless and very loosely interwoven next to the intermediate layer becoming progressively more interwoven and colored outward, finally forming a rind of very compactly interwoven dark colored hyphae through which the ostioles of the perithecia open.

CORDYCEPS RAVENELII Berk. & Curt.

This is also a rare species. It was described by Berkeley (1) in 1857 from a collection made by Ravenel in South Carolina. Ravenel issued it in his Fungi Car. IV : 28. It has been reported from South Carolina, North Carolina, Tennessee, Kentucky, Iowa, Pennsylvania and New Hampshire. In 1940 it was discovered in a number of localities in Michigan by A. H. Smith and the writer on grubs of the June beetle. The first collection was obtained on June 4, and the clavae proved to be immature. However, they were planted in the writer's garden where they continued to develop. It was noted that the developing perithecia appeared to

break through a cortical layer. The perithecia of this species have been described as free, superficial or partially immersed. Sections through the fertile portion of a clava (FIG. 2, *A–C*) show that a considerable portion of it is a central cylinder consisting of light colored, narrow, longitudinal hyphae. Surrounding this is an intermediate layer, 30–36 μ thick, of densely interwoven hyphae. Arising from this and growing outward at right angles are numerous dark, brown parallel hyphae which form a very dense layer about 60 μ thick. The perithecia arise at intervals from the intermediate layer and apparently as they develop push through the outer layer. Where the perithecia are closely associated, the outer layer is torn loose from the intermediate and sloughs off (FIG. 2, *B*). Since the perithecia finally reach a length up to 480 μ, they appear to be superficial even where the outer layer remains (FIG. 2, *C*).

The following description is drawn from fresh specimens of the Michigan collections:

Clavae 5–9.5 cm. long, dark chocolate-brown, club-shaped, the fertile portion occupying the upper portion of the clava, 2.5–4 cm. long, 4–7 mm. thick, the apices obtuse to acute, sometimes free from perithecia, the stipes 2–3 mm. thick; perithecia at first nearly hemispherical, finally cylindric, rounded above or somewhat narrowed, 348–480 $\times$ 240–360 μ, blackish brown, at first embedded, finally becoming almost or entirely free; asci somewhat clavate, 240–312 $\times$ 8–10 μ; ascospores slightly narrowing at the ends, 192–255 $\times$ 2–3 μ, hyaline, multiseptate, the cells 20–30 μ long, only slightly segmenting.

From larvae of "June beetle," in woods, Milford, Michigan, E. B. Mains and A. H. Smith, June 4, 1940 (*5062*), June 5, 1941 (*5325*); Dexter, Michigan, June 14, 1940, A. H. Smith (*15100*); Waterloo, Michigan, June 26, 1940, A. H. Smith (*15131*); Pinckney, Michigan, June 17, 1940, E. B. Mains (*5090*); Ann Arbor, Michigan, July 3, 1940, A. H. Smith (*15174*).

Species of *Cordyceps* are commonly grouped according to whether their perithecia are embedded or superficial. Some species, as for example *Cordyceps militaris,* have been placed in both groupings. However, in *C. militaris,* the perithecia are embedded in a peripheral layer of loose hyphae without an outer compact rind.

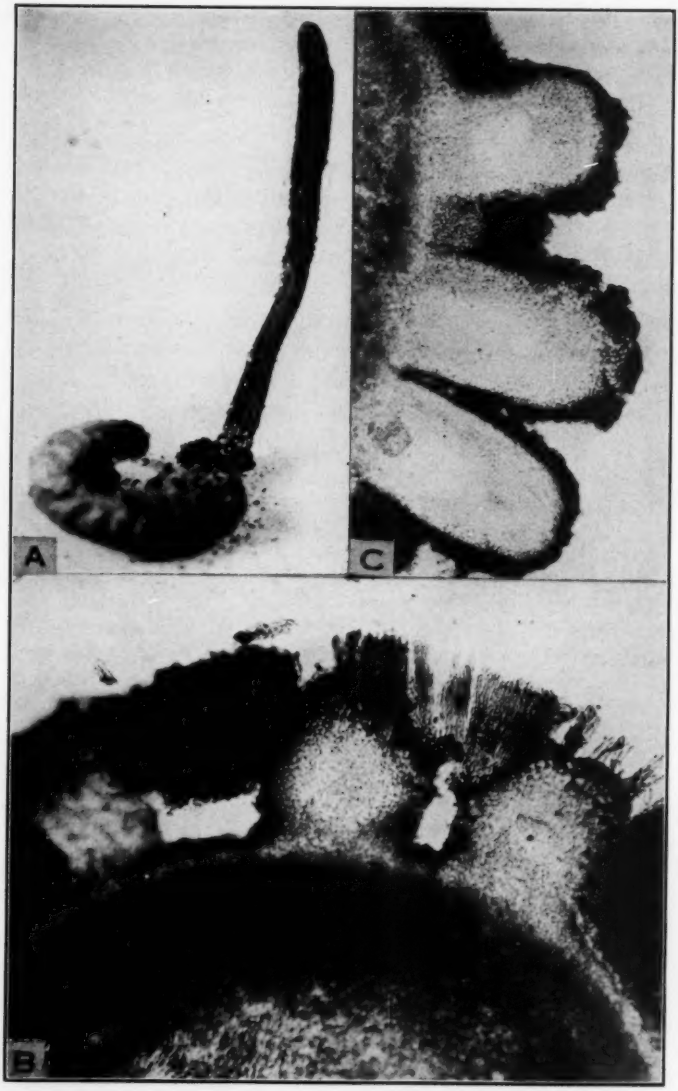

FIG. 2. *Cordyceps Ravenelii.*

Upon drying or in age, the tissue between the perithecia shrinks leaving most of each perithecium exposed and projecting. The dense outer rind of *C. stylophora* prevents this. *Cordyceps michiganensis, C. paludosa* and *C. superficialis* unquestionably have superficial free perithecia in all the collections examined. These species are very rarely collected and the available material is rather limited. From such observations as it has been possible to make it seems probable that the development of these species is similar to that of *Cordyceps Ravenelii;* the peripheral layers being very thin and the perithecia emerging very early in their development.

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EXPLANATION OF FIGURES

FIG. 1. *Cordyceps stylophora. A,* clava showing swollen fertile portion and acuminate sterile apex (1.5 ×); *B,* portion of clava, showing exudation of spores (60 ×); *C,* portion of cross section of immature clava showing central cylinder covered by intermediate layer and cortical layer containing perithecia (150 ×).

FIG. 2. *Cordyceps Ravenelii. A,* clava arising from June beetle larva (natural size); *B,* cross section of fertile portion of clava showing portion of central cylinder covered by intermediate layer from which perithecia and cortical layer arises; developing perithecia have torn portions of the cortical layer loose (105 ×); *C,* portion of cross section of clava showing half developed perithecia projecting above the cortical layer (105 ×).

OBSERVATIONS ON A NEW SPECIES OF CLADOCHYTRIUM

ARTHUR B. HILLEGAS

(WITH 40 FIGURES)

During the course of an investigation of the chytrid flora of Van Cortlandt Lake in New York City, an interesting species of *Cladochytrium* was isolated from collections of water and decaying vegetation. Although at first the fungus appeared to be similar to *Cladochytrium tenue* as described by Nowakowski (**1877**) further investigation brought out significant differences which indicated that an intensive study ought to be made. Chiefly because of its exceptionally coarse and extensive rhizomycelium and the presence of numerous, non-septatę, intercalary, spindle-shaped enlargements, it became apparent that it is not identical to any of the known *Cladochytrium* species or any other closely related Cladochytriaceae, and the creation of a new species seems warranted. Since the fungus is characterized by an unusually coarse rhizomycelium, the following name is proposed:

Cladochytrium crassum sp. nov.

Fungus saprophyticus; rhizomycelio copioso atque extenso, maxime crasso cum permultis tumoribus intercalaribus, non septatis, fusiformibus vel globosis, 3.85 × 15–18 × 30 μ, partibus tenuissimis 1.5 μ diametro, trabeculis apparentibus. Rhizoidibus 0.5–1.1 μ diametro in rhizomycelio variatim locatis. Zoosporangiis terminalibus aut intercalaribus, raro proliferatis, sine apophysate, variatim formatis sed plerumque sphaericis ad aliqua ex re pyriformia, 11 × 20–30 × 43 μ. Tubula exeunte nonnumquam usque ad 27 × 74 μ, papilla aut poro, plerumque una, deliquescente. Zoosporis intra zoosporangium disiunctis, immotili cumulo emergentibus, limo involutis, orifice tubulae exeuntis quiete aliquamdiu manentibus, mox acriter moventibus atque enatantibus. Zoospora hyalina, sphaerica ad minime pyriformem, interdum amoeboidea, 4.9–6 μ diametro cum hyalino globulo maxime refractivo 2–2.75 μ diametro, flagello posteriore 25–35 μ, rapide et emicatim enatante. Spora perdurante sphaerica (9.35–23 μ diametro) vel fusiformi (10 × 26 μ–14.8 × 23.1 μ), pariete 1.5 μ crasso, subfusco colorato, germinatione incomperta.

Rhizomycelium well developed, extensive, coarse, with numerous intercalary, non-septate, fusiform to globose swellings 3.85 × 15–

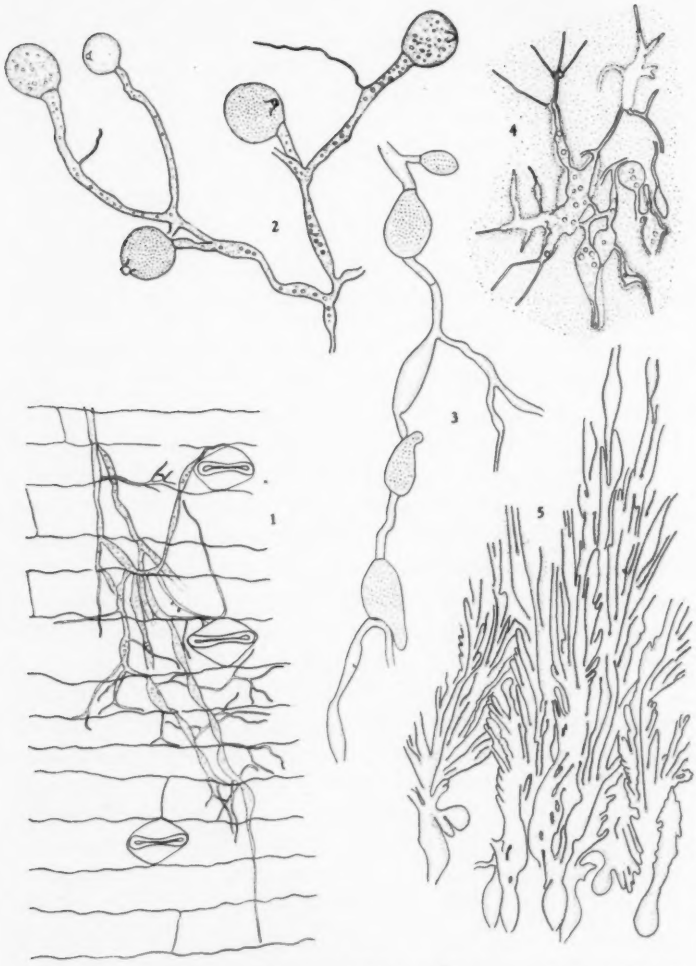
18 × 25 μ, the tenuous portions as little as 1.5 μ in diameter, with trabeculae. Rhizoids 0.5 μ to 1.1 μ in diameter originating at various places along the rhizomycelium. Zoösporangia terminal or intercalary, seldom proliferating, non-apophysate, variously shaped, commonly spherical to slightly pyriform, 11 × 20–30 × 43 μ. Exit tube occasionally to 27 × 74 μ, papilla or pore, usually single, deliquescent. Zoöspores delimited within zoösporangium and emerging in a non-motile mass enveloped in slime, which remains at the mouth of the exit tube a few minutes before the zoöspores become active and swim away. Zoöspore hyaline, spherical to slightly pyriform, occasionally amoeboid, 4.9–6 μ in diameter with a clear highly refractive globule 2–2.75 μ in diameter, posterior flagellum 25–35 μ, swimming rapid and darting. Resting spore spherical (9.35–23 μ diameter) to fusiform (10 × 26 μ–14.8× 23.1 μ), wall 1.5 μ thick, light brown in color, germination unknown. Saprophytic on decaying vegetation from Van Cortlandt Lake, New York City.

APPEARANCE OF THE FUNGUS IN CULTURE

The fungus was first observed in dechlorophyllized corn leaves which had been used to bait the original collections of pond water. From this mixed culture unifungal cultures were established on bleached corn leaves and non-waterproof cellophane, and in pure culture upon 0.5 and 3 per cent plain agar. The methods employed in isolating these cultures are essentially those which have been employed by Couch (**1939**) and Cox (**1939**).

For a life history study of *Cladochytrium crassum* bleached corn leaves proved to be the best substratum, because on it the thallus develops normally and abundantly. The rhizomycelium characterized by its conspicuous, irregular, globose, or spindle-shaped swellings may grow intramatrically (FIG. 1) and partially or entirely extramatrically, frequently extending into the surrounding water and giving a distinctly fuzzy appearance to the margin of the corn leaf. Usually the rhizomyceloid development of the thallus is well established before the terminal (FIG. 2) or intercalary (FIG. 3) zoösporangia are formed. Although zoösporangia are distributed throughout the whole substratum they are particularly abundant about the vascular system, especially in old cultures.

Another substratum used successfully for maintaining cultures of the fungus was non-waterproof cellophane, a substratum introduced into chytrid work by Haskins (**1939**). Although this is



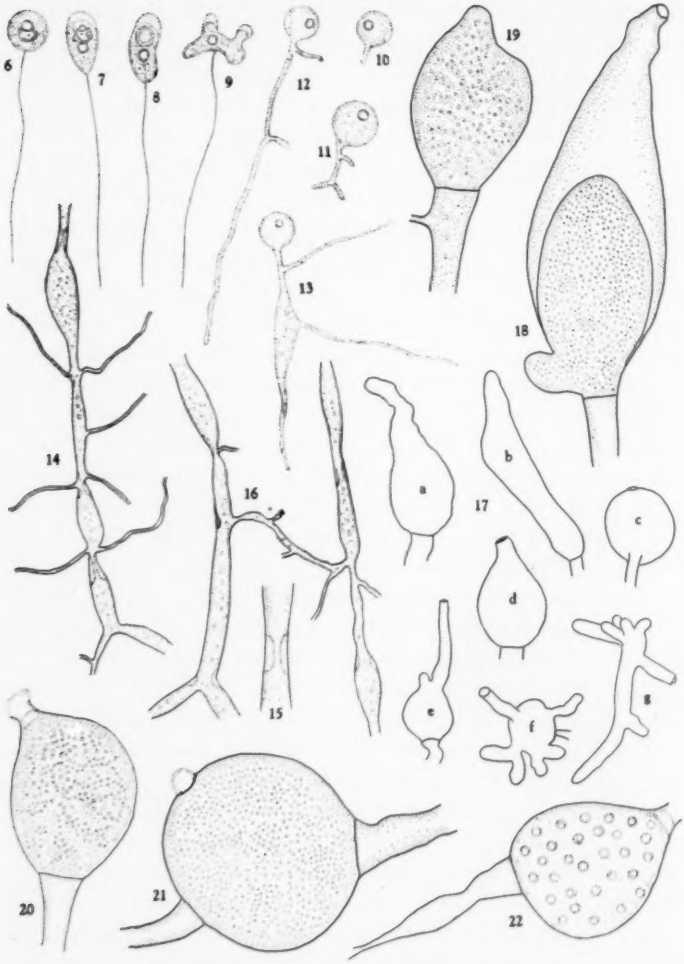
FIGS. 1–5. 1, thallus growing in dechlorophyllized corn leaf, × 296; 2, rhizomycelium with terminal zoösporangia, × 296; 3, intercalary zoösporangia, × 296; 4, young thallus growing upon cellophane, × 1187; 5, old thallus scraped from cellophane showing anastomoses between branches.

excellent for culturing purposes it is not desirable for observation of the fungus because of the similar optical properties of the fungus and the cellophane, and the reduced and distorted thallus that is produced. Usually about ten days after inoculation white patches or mats of the fungus on the cellophane are to be observed with the unaided eye. Instead, however, of expanding widely on the cellophane as it does on the bleached corn leaves, the fungus displays an abundant but compacted and distorted growth on the surface of the cellophane radiating centrifugally from the point of infection (FIG. 4). Immediately upon germination of the zoöspore, branching and enlargement and anastomosis of the germ tube occurs (FIG. 4). The delicate tips of the rhizomycelium lie plainly on the surface of the cellophane but the proximal portion is less clearly defined and appears to be partially enveloped, possibly because of the swelling of the cellophane induced by enzymic action. An old empty thallus scraped from the cellophane, flat and rigid, shows what an intricate labyrinthine pattern is formed by the reduced growth and the anastomoses between branches of the thallus (FIG. 5). The zoösporangia produced on cellophane, unlike the vegetative growth, appear to be quite normal except in places where they have developed so abundantly that they are packed and squeezed together into irregular shapes.

Cladochytrium crassum has been grown on 0.5 and 3 per cent plain agar, and on these media the thallus development is quite normal. The rate of growth is nevertheless slow requiring two weeks to acquire a colony 6 mm. in diameter on 3 per cent agar. In such cultures growth takes place both deep within and on the surface of the medium. Zoösporangia are developed but zoöspores have not been observed to be liberated from the zoösporangia at the concentrations of agar thus far employed. Further experiments will determine the relative suitability of various agar media.

DEVELOPMENT OF THE FUNGUS

The zoöspore of *C. crassum* is typical of the genus *Cladochytrium*. The single highly refractive globule is hyaline in contrast to the golden-red refringent globule of *Cladochytrium replicatum* (Karling, **1931**), *Cladochytrium Nowakowski* (Sparrow, **1931**), and *Cladochytrium polystomum* (Zopf, **1884**). The zoö-



FIGS. 6–22. 6, motile zoöspore, × 1175; 7–9, amoeboid zoöspores, × 1175; 10, early stage in germination of zoöspore, × 1175; 11, 12, branching of the germination tube, × 1175; 13, initial swelling on the germ tube, × 1175; 14, intercalary swellings of mycelium and trabeculae, × 572; 15, enlarged view of trabecula, × 1175; 16, anastomosis between two branches of rhizomycelium, × 572; 17 *a–g*, various sporangial shapes, × 382; 18, proliferation of a zoösporangium, × 1175; 19, early stage in the development of a zoösporangium with exit tube forming, × 1175; 20, late stage in the dispersal

spores are spherical to slightly pyriform 4.9 to 6 μ in diameter, hyaline, occasionally with small vacuoles, with a posteriorly attached flagellum 35 μ long which compares favorably with those reported for *Cladochytrium tenue* (Nowakowski, **1877**). A conspicuous spherical highly refractive globule 2–3 μ in diameter is contained in each spore usually in a fairly central position (FIG. 6, 7). Adjacent to the refractive globule is a clear spherical body bordered by a denser material which appears to be the nucleus surrounded by a nuclear cap.

The zoöspore swims forward in the usual jerky manner of a chytrid, darting from one obstruction to another. This active swimming stage of the zoöspore is frequently interrupted by an amoeboid condition of varying duration in which the active zoöspore comes to rest, becomes amoeboid, and then weakly creeps about trailing its inactive flagellum (FIG. 8, 9). During the amoeboid phase the nucleus and nuclear cap material remain closely associated with the highly refractive globule in the dense cytoplasm. The optically homogeneous pseudopodia which extend out in the direction of movement are definitely less dense than the central portion of the zoöspore.

After a swimming period of a few minutes to several hours the zoöspore comes to rest on the substratum, rounds up preliminary to germination, and either drops or retracts its flagellum. The protoplasm now assumes a greyish hue and all the structures in the zoöspore that have been so clearly defined in the hyaline protoplasm now become indistinguishable except the highly refractive globule. Within a few minutes the germ tube emerges from the surface of the zoöspore as a small papillate structure which elongates into a fine hyaline filament 2 μ in diameter (FIG. 10) and branches either near the zoöspore (FIG. 11) or at some distance away (FIG. 12). As the germ tube elongates, gradual vacuolation of the zoöspore takes place until it is devoid of any protoplasm except the highly refractive globule and this too soon disintegrates. Very soon afterwards intercalary enlargements make their appear-

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of highly refractive substance in the protoplast; deliquescence of exit papilla, × 1175; 21, intercalary sporangium in granular stage with exit pore, × 1175; 22, mature zoösporangium with refractive substance localized in each zoöspore initial, × 1175.

ance in the germ tube (FIG. 13), the initial stages of the spindle-shaped swellings which are so strikingly characteristic of the thallus. The germination therefore is not different in important respects from that reported for other Cladochytriaceous species.

The germ tube represents the rudimentary rhizomycelium which by its growth, branching and rebranching forms a very extensive and complicated thallus. The most striking feature of the thallus is its spindle-shaped, or less frequently, irregular (FIG. 1) or globose swellings. They may attain a diameter of 18.7 μ while the more tenuous portions are less than 3 μ in diameter and the extremely delicate tips are never more than 1 μ. A hyaline protoplasm in which are dispersed highly refractive globules fills the young thallus while the older ones which have been differentiated into vegetative and reproductive structures are devoid of protoplasm in the filamentous portion. The protoplasm appears to be drained into the incipient zoösporangia although occasionally extremely vacuolated protoplasm may remain in the filament where it soon disintegrates, possibly indicating that not all of the protoplasm is utilized in the formation of the zoösporangia.

The spindle-shaped swellings of *Cladochytrium crassum* are not septate as they have been shown by Nowakowski (**1877**) for *Cladochytrium tenue*. This is one of the fundamental differences between these two species. Furthermore the relationship between the spindle organ and the more tenuous portion of the rhizomycelium is another point of contrast. The figures of Nowakowski (**1877**) indicate that the spindle organs of *C. tenue* are of fairly uniform size and shape and that they taper rapidly to the tenuous portions of the rhizomycelium which likewise maintain uniformity in diameter. On the other hand, *C. crassum* exhibits no uniformity either in size and shape of its spindle-shaped swellings or in the diameter of its tenuous portions. Frequently one swelling may follow another in catenulate fashion without the interval between the swellings becoming sufficiently reduced to be called tenuous, thus presenting a thick appearing mycelium (FIG. 2). This same comparison might be made with *Cladochytrium replicatum* (Karling, **1931**, **1935**, **1937b**) and *Cladochytrium Nowakowski* (Sparrow, **1931**). The type of swelling found in this new species is in most respects similar to those of *Amoebochytrium*

rhizidioides (Zopf, **1884**), *Cladochytrium cornutum* (de Wildeman, **1896**), *Nowakowskiella ramosum* (Butler, **1907**), *Nowakowskiella elegans* (Matthews, **1928**; Sparrow, **1933**), *Nowakowskiella endogena* (Domjan, **1935**), *Physocladia obscura* (Sparrow, **1933**) and *Septochytrium variabile* (Berdan, **1939**).

Another distinguishing characteristic of the rhizomycelium of this species is the trabeculae or thickenings within the filamentous portion of the thallus. These bands may be so thick as to leave only a small pore through which the protoplasm can flow or they may only partially surround the mycelium and scarcely retard the flow of the protoplasm (FIG. 14, 15, 16). These trabeculae are usually located at a constriction in the mycelium or just at the expanding portion of the intercalary swelling. Partial thickenings are located at various places along the mycelium but most often at the junction of the branches or rhizoids or at other partial obstructions in the filament (FIG. 16). Until the present time thickenings of this type among chytrids have been reported only in *Septochytrium variabile* (Berdan, **1939**). Occasionally anastomosis between the branches of the rhizomycelium may occur (FIG. 16), but this does not appear to serve any particular function.

The zoösporangia are either terminal or intercalary in the rhizomycelium as has already been shown (FIG. 2, 3, 21) and exhibit great diversity in size and shape and in the length of exit tube. They are typically globose or pyriform measuring 11 × 20 μ–30 × 43 μ (generally 34 μ in diameter) while others may be irregular or spindle-shaped. A few of the infinite number of shapes which the zoösporangia may assume are shown in figures 17*a–g*. The zoöspores may be discharged through a long and slender exit tube up to 46 μ in length and 3.5–5 μ in width, or by means of a very short exit papilla, or by simply a pore. More than one exit tube seldom occurs (FIG. 17*g*) and although as many as three of them have been observed only one has ever been functional.

The zoösporangia originate as terminal or intercalary swellings which have become delimited from the vegetative portion of the thallus by cross-walls (FIG. 19). Whether or not the protoplasm of the rhizomycelium is entirely used up in the formation of the zoösporangia was not determined. It is observed, however, that the protoplasm is absent from the older portions of the mycelium

and often in those parts adjacent to the sporangia. This may be the result of drainage of the protoplasm into the sporangia or possibly degeneration of it. Proliferation of the sporangium occurs infrequently (FIG. 18).

The subsequent enlargement and maturation of the zoösporangium is accompanied by certain changes in the protoplast. In the initial enlargement are numerous small highly refractive globules scattered throughout the hyaline protoplasm (FIG. 19, 20). These increase in number as the incipient sporangium enlarges, but just before the cleavage of the protoplast into zoöspores, these globules become uniformly dispersed throughout the hyaline protoplasm in the form of minute granules (FIG. 21), imparting a finely granular appearance to it. This dispersion allows equal amounts of the highly refractive substance to be included in each of the zoöspore initials at cleavage. The optical homogeneity of the protoplasm makes it impossible to follow the cleavage stages in the protoplast. Zoöspore delimitation, however, follows the highly dispersed phase of the refractive material because gradually the refringent substance, by fusion of the minute particles, becomes localized to form the highly refractive globule of the zoöspore (FIG. 22). The changes that occur in the protoplast have for the most part been described for many of the rhizidiaceous and cladochytriaceous fungi by Karling, Couch, Berdan, Hillegas and others.

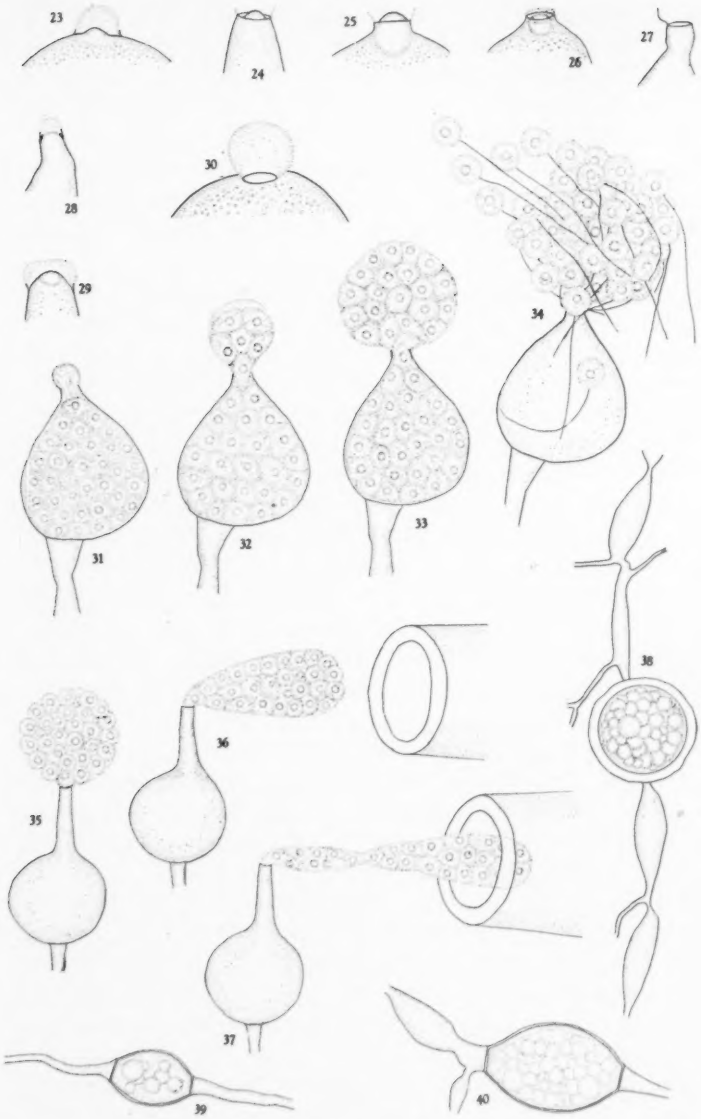
The discharge of the zoöspores of *Cladochytrium crassum* may occur through an exit tube, a papilla (FIG. 2), or a pore (FIG. 21). If a tube is formed it begins as a bulge on the surface of the sporangium (FIG. 19) and elongates rapidly attaining in one instance a length of 14 μ in one hour. Except under unfavorable circumstances long tubes are not formed. This development occurs before the highly refractive material becomes dispersed throughout the protoplast. The preliminary steps involved in the preparation of the opening through which the zoöspores are to be discharged are fairly complicated. At the tip of the exit tube or papilla, or in the case of the simple pore, on the surface of the sporangium itself, a bulge becomes evident which appears to be caused by the separation of inner and outer layers of the sporangial wall. In the space thus formed between the two layers a clear substance forms which might conceivably be the products of the

deliquescence of the outer layer. When this outer layer eventually disappears leaving only traces in the form of a ring (FIG. 24, 25, 26, 29) the hyaline substance also soon disappears leaving the inner membrane of the wall exposed. This membrane similarly has a bulge (FIG. 23, 24, 25, 26) directly inside of which is evident a second spherical-shaped mass of viscid substance (FIG. 25, 26). Upon the rupturing of this inner membrane, this viscid substance serves as a temporary plug and is pushed out ahead of the zoöspores (FIG. 31).

Zopf (**1884**) figured a plug-like structure in the exit tube of *Amoebochytrium rhizidioides* and Ward (**1939**) in *Rhizophlyctis rosea* has shown a cushion-like plug in the mouth of the exit tube which may correspond to the substance found between the outer and inner membranes in *Cladochytrium crassum.* For *Cladochytrium hyalinum* Berdan (**1941**) showed a cushion of hyaline substance in the mouth of the exit tube but outside the inner membrane. This substance she believes to serve as a plug preceding the discharge of the zoöspores and to later surround the released zoöspores. In *C. crassum,* on the other hand, the substance which corresponds to the cushion in *C. hyalinum* entirely disappears before the zoöspores emerge and it is the viscid substance beneath the inner membrane which precedes the escaping zoöspores and partially or possibly completely surrounds them upon their discharge.

In two instances empty blister-like protuberances were seen (FIG. 30) resembling a vesicle extruding from the pore, but in neither case did the protoplasmic contents flow into this such as has been described by Sparrow (**1932**) in the discharge of the spores of *Physocladia obscura.*

Following the breaking of the membrane at the mouth of the exit tube, the clear substance which up to this time has remained just below the bulging membrane (FIG. 25, 26) appears to function as a plug which restrains the zoöspores from escaping (FIG. 22). As the zoöspores emerge pushing the viscid material before them (FIG. 31), this clear substance forms a cap over the top of them (FIG. 32) which gradually thins out over the zoöspore mass as the mass enlarges, but because of its hyaline nature it soon becomes indiscernible. Ultimately the entire mass of zoöspores flows out



FIGS. 23–40.

of the sporangium and remains at the mouth of the exit tube as a spherical mass of amoeboid bodies so closely pressed together that they assume polygonal shapes (FIG. 33). The zoöspores now round up, then gradually become active and with their flagella moving weakly they swarm about within a limited space before they become dispersed and swim away (FIG. 34).

As the figures and description indicate, there is some evidence in support of the view that there is a membrane or vesicle surrounding the zoöspores. Immediately after discharge the zoöspores appear to have a cap of clear fluid which at some times appears to act like a membrane. For example the restrained swarming of the zoöspores at the mouth of the exit tube prior to their liberation into the surrounding medium might be considered a proof of the existence of a membrane. This limitation of swarming cannot have been caused by a bacterial slime because it has been observed in cultures which have been thoroughly washed. Furthermore, when these zoöspores after a minute begin to swim away they do not, as in *Endochytrium operculatum* (Karling, **1937a**), break forth in all directions, but emerge usually at one, occasionally at two, spots from the zoöspore mass, a behavior that could be the result of the presence of some limiting membrane which ruptured at one or two places thus permitting the liberation of the zoöspores. The zoöspores are well bound together by some means.

FIGS. 23–40. 23, early stage in the deliquescence of exit pore, × 1134; 24, exit tube with remnants of outer wall and bulge showing on inner membrane, × 1134; 25, exit papilla on maturing zoösporangium; fragments of outer wall bulge on inner membrane with globule of viscid substance below, × 1134; 26, oblique view of exit papilla; fringe formed by old outer wall, × 1134; 27, exit tube with inner membrane ripped off; 28, thickened ring formed at contact of outer wall and inner membrane of exit tube, × 1134; 29, exit tube with outer wall partially ripped thus showing inner membrane, × 1134; 30, vesicle at mouth of exit pore, × 1134; 31, zoöspore discharge; viscid fluid flowing from exit papilla, × 1134; 32, viscid substance spreading out over emerging zoöspores, × 1134; 33, later stage of zoöspore discharge, viscid substance not visible, × 1134; 34, zoöspores fully discharged and swimming away, × 1134; 35, zoöspore mass at mouth of exit tube; 36, zoöspore mass being stretched towards micropipette; 37, mass of zoöspores showing extreme elasticity; 38, intercalary thick-walled resting spore, × 1134; 39, 40, thin-walled intercalary resting spores, × 1134.

In spite of this evidence, however, I do not believe that there is any membrane around the zoöspores of *C. crassum* as in *Pythium* or *Physocladia obscura*. Even after repeated and long observation I have been unable to discern any traces of such a vesicle. Sparrow (**1932**) reported *Physocladia obscura* as possessing a vesicle within which the zoöspores swarmed before they were released. The zoöspores of *C. crassum* swarm no longer than one minute thus making the existence of such a vesicle highly improbable in this form. It is my belief, therefore, that the zoöspores of *C. crassum* are embedded in a matrix or surrounded by a slime which momentarily retards the free swimming of the escaped zoöspores. The extreme viscidity of the matrix or slime was demonstrated when making a unifungal culture. A pipette placed near the mass of discharging zoöspores (FIG. 35) could stretch the mass of zoöspores away from the mouth of the exit tube into a long strand (FIG. 36, 37) without separating the mass, and when suction was released the mass snapped back elastically into its original shape and position.

RESTING SPORE

Resting spores form rather abundantly in old cultures to which fresh water has not been added for at least two weeks. They exhibit characteristics similar in most respects to those reported for other members of the genus *Cladochytrium*. They are intercalary, spherical (FIG. 38) or fusiform (FIG. 39, 40) with a smooth hyaline wall 1.5–2 μ in thickness. They appear to form within the intercalary swellings and are cut off from the rhizomycelium by septa. The contents of the resting spores consist of several large highly refractive globules that fill the interior of the spore. Resting spores have been reported for six of the recognized species of *Cladochytrium: C. graminis* (Büsgen, **1887**) which have thick light brown resting spore walls; *C. replicatum* (Karling, **1935**) (*C. Nowakowski* Sparrow, **1931**) with smooth and rough hyaline walls; *C. caespitis* (Griffon and Maublanc, **1910**) whose resting spores have smooth, slightly yellowish walls 2–5 μ thick; *C. irregulare* (de Wildeman, **1895**) which has terminal or intercalary resting spores; *C. viticolum* (Prunet, **1894**) with resting

spores having thick, smooth walls; and *C. hyalinum* (Berdan, **1941**) which has smooth, thin walled resting spores.

It is evident from this that the general morphological character of the resting spore of *C. crassum* is for the most part similar to that of other species of *Cladochytrium*. The germination of the resting spore has not been observed.

DISCUSSION

The fundamental characteristics on the basis of which the new species has been created make the fungus easily distinguishable from its closest relative *Cladochytrium tenue*. The new species displays a coarse rhizomycelium with intercalary non-septate swellings and prominent trabeculae. Resting spores have also been observed. In *Cladochytrium tenue,* however, the rhizomycelium is tenuous except for the spindle organs which are bi-cellular instead of non-septate, and no trabeculae nor resting spores have been reported thus far.

SUMMARY

1. A new species of *Cladochytrium* has been isolated in unifungal culture on dechlorophyllized corn leaves and non-waterproof cellophane, and in pure culture on 0.5 and 3 per cent plain agar.

2. The development and structure of the fungus has been described.

3. The specific name *crassum* has been proposed for this new species because of its coarse rhizomycelium.

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DICTYOSTELIUM MINUTUM, A SECOND NEW SPECIES OF SLIME MOLD FROM DECAYING FOREST LEAVES [1]

KENNETH B. RAPER [2]

(WITH 4 FIGURES)

INTRODUCTION

Species of the Dictyosteliaceae, a group of pseudoplasmodium-forming slime molds, are generally distinguished by one or more of the following characters: (1) the color of the spore masses, or *sori*, (2) the form of the fruiting structures, or *sorocarps*, and (3) the maximum dimensions of such sorocarps. Of these characters, the latter is the most variable and, in general, the least satisfactory. Nevertheless, sorocarp size provides a reasonable basis for species separation and description if adequate safeguards are taken to establish and maintain optimum cultural conditions. To this end it is essential to cultivate members of this group with the same bacterial associates, and, insofar as possible, to standardize critical environmental factors at favorable and reproducible levels. Such factors include (1) the nutrient composition of the substratum, (2) the moisture content of the air within the culture chambers, (3) the temperature of incubation, and (4) the pH of the host bacterial colonies (Raper, **1937**, **1939**, **1940b**). Upon observing these precautions, whenever a particular isolate consistently shows marked differences in sorocarp size from known species in culture, and likewise differs from the description of previously published species,

[1] The slime mold described was isolated and studied while the writer was employed in the Division of Soil Microbiology, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

[2] Microbiologist, Northern Regional Research Laboratory. This is one of four regional laboratories authorized by Congress in the Agricultural Adjustment Act of 1938 for the purpose of conducting research to develop new uses and outlets for agricultural commodities. These laboratories are administered by the Bureau of Agricultural Chemistry and Engineering of the U. S. Department of Agriculture.

the designation of such a form as new appears warranted. Such has been the writer's experience. Upon three occasions and from widely separated localities a *Dictyostelium* has been isolated from forest litter which invariably produces smaller sorocarps than any known member of the genus. Because of the diminutive character of its sorocarps, the species is designated as follows.

TECHNICAL DESCRIPTION [3]

Dictyostelium minutum sp. nov.

In culturis artificiosis, sorocarpis perminutis, solitariis vel gregariis, plerumque 500–850 μ altis, interdum majoribus, saepe minoribus, frequenter ramosis; sorophoris ex hyalinis griseo-albis, rectis, basi 12–18 μ, apicem versus 2.5–6 μ in diam. paulatim attenuatis, supra flexuosis, parte basali excepta e strato uno cellularum compositis; soris ex hyalinis lacteo-albis, rotundato-apiculatis, plurimum 75–125 μ in diam., aliquando majoribus; sporis ellipticis, hyalinis, 5–7 μ longis, 3.0–3.5 μ latis.

Ex foliis putrescentibus in silvis deciduis isolatum, Virgnia, Massachusetts, et Maryland.

Cultivated upon hay-infusion, dung-infusion, and weak peptone agars in association with *Escherichia coli, Pseudomonas fluorescens, Serratia marcescens, Bacillus megatherium,* and *Bacillus firmus* at temperatures between 20 and 25° C., sorocarps diminutive, solitary or gregarious, commonly 500 μ–850 μ in height, occasionally larger, often smaller, frequently branched; sorophores colorless to grayish white, erect, 12 μ–18 μ in diameter at the base, tapering gradually to 2.5 μ–6 μ in diameter above, terminal region flexuous, commonly consisting of a single tier of cells except in basal portion; sori colorless to milk-white, rounded-apiculate, commonly 75 μ–125 μ in diameter, occasionally larger; spores elliptical, hyaline, 5 μ–7 μ × 3.0 μ–3.5 μ.

Isolated from leaf mould from deciduous forests. Virginia, Massachusetts, and Maryland.

ISOLATION AND CULTURE

The type culture of *Dictyostelium minutum,* No. V–3, was isolated in October 1937 from a sample of forest litter collected from a hardwood forest near Vienna, Virginia. The site of collection

[3] The writer is indebted to Edith K. Cash, Bureau of Plant Industry, for preparing the Latin diagnosis.

was near a small stream, and the flora in the immediate vicinity consisted of maples, ash, alder and deciduous ferns. These forms, together with oaks and beeches from the adjacent slopes, con-

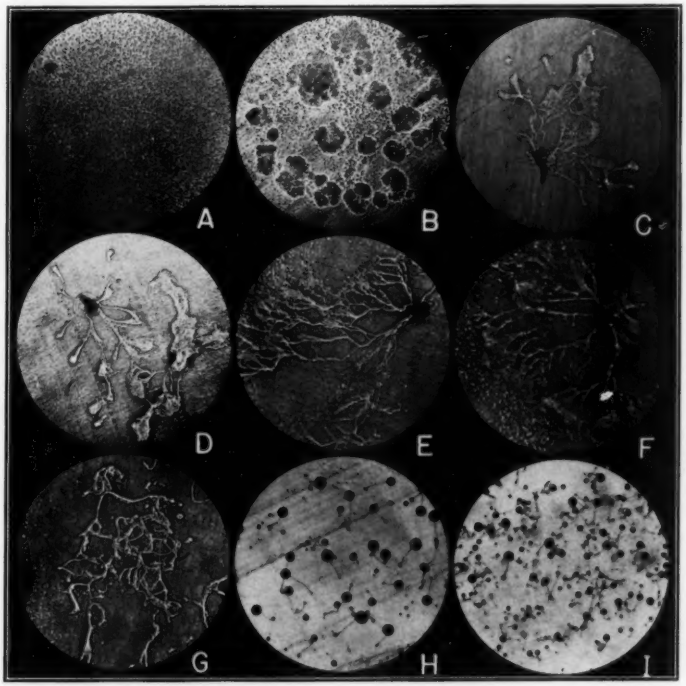

FIG. 1. *Dictyostelium minutum* growing in association with *Escherichia coli* at 22°–23° C.; figures with exception of I taken from 0.1% peptone agar plates. *A*, close of vegetative stage, myxamoebae unoriented; *B*, early fruiting stage, aggregates not showing converging streams; *C–F*, pseudoplasmodia showing conspicuous centers and converging streams of myxamoebae; *G*, pseudoplasmodium not showing definite center of aggregation; *H*, mature sorocarps on 0.1% peptone agar; and *I*, mature sorocarps on hay-infusion agar. *A–G*, × 35; *H–I*, × 10.

tributed to the leaf mould layer. The material from which the slime mold was isolated remained fairly most at all times and consisted of decomposing leaf fragments together with the topmost layer of underlying soil. From the same sample of approximately

40–50 grams, cultures of *Dictyostelium mucoroides* Brefeld (**1869**), *Polysphondylium violaceum* Brefeld (**1884**), and *P. pallidum* Olive (**1901**) were likewise secured.

The slime molds were isolated as follows: A portion of the sample was macerated in a clean mortar with approximately ten volumes of sterile water and the resulting suspension streaked upon dilute hay-infusion agar[4] plates. These were subsequently incubated at 22° C. ±. Beginning on the third day following inoculation, the plates were examined daily. Pseudoplasmodia characteristic of the Dictyosteliaceae appeared on the fourth day and by the following morning had developed into typical but relatively small sorocarps of *Dictyostelium mucoroides* and *Polysphondylium violaceum*. On the sixth day sorocarps of *P. pallidum* were likewise evident. The three species were isolated directly in pure-mixed culture with *Escherichia coli*. Meanwhile, in a single plate, small pseudoplasmodia appeared which subsequently developed into diminutive white-headed sorocarps. These were almost completely masked by an overgrowth of Mucors and other fungi. After removing this covering as carefully as possible, minute quantities of unaggregated myxamoebae and accompanying "host" bacteria were removed with a small loop and streaked upon fresh plates of dilute hay-infusion agar. By repeating this procedure the fungi were soon eliminated and the slime mold was eventually obtained in pure-mixed culture associated only with a single species of bacteria upon which it fed. In addition to dilute hay-infusion agar, subsequent transfers were made upon full-strength hay-infusion, dung-infusion, and weak peptone agars containing either 0.05 per cent or 0.1 per cent of this nutrient. The slime mold has been grown continuously upon these media since the date of its isolation, being frequently shifted from one to another.

In the light of previous experience (Raper, **1939**, **1940b**) environmental factors known to influence processes of growth and development in the Dictyosteliaceae have been controlled as accurately as deemed necessary. Culture media have regularly been adjusted to an initial pH of 6.0 and quantities of phosphate buffers ($Na_2HPO_4 \cdot 12H_2O$ and KH_2PO_4) have been added whenever

[4] Hay infusion agar as earlier described (Raper, **1937**) diluted to one fourth its original nutrient strength.

necessary to prevent any appreciable deviation from this level. Cultures for examination have been grown in Petri plates consisting of close fitting valves, insuring the maintenance of a saturated or near saturated atmosphere within the culture chambers throughout the period of growth and sorocarp development. Incubation temperatures have ranged from 20°–25° C., but have generally been maintained at approximately 22°–23° C. The latter temperature appears to be optimum for the species.

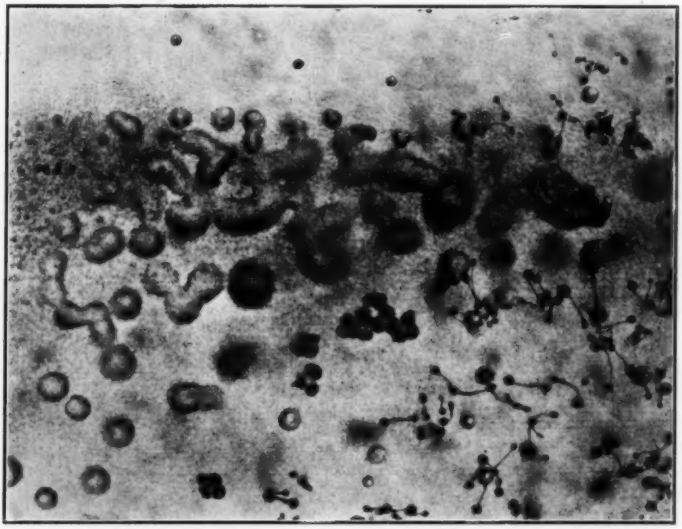

FIG. 2. Fructification of *Dictyostelium minutum* in association with *Escherichia coli* upon hay-infusion agar. Upper left, initiation of fruiting phase; left center, pseudoplasmodium formation by the blocking-out of masses of myxamoebae; right center, early stages in sorocarp formation; lower right, mature sorocarps. × 10.

The bacterium accompanying *Dictyostelium minutum* at the time of its isolation and subsequently associated with it in the writer's stock cultures has been identified as *Bacillus firmus* Bredemann and Werner.[5] In association with this host organism the diminu-

[5] Determination by Ruth E. Gordon and Nathan R. Smith, Division of Soil Microbiology, Bureau of Plant Industry, U. S. Department of Agriculture.

tive size and character of the sorocarps remained essentially constant (1) upon each of the above media and (2) throughout the period of observation which covered more than a score of culture generations. The number of sorocarps, however, was consistently much greater upon the richer culture media. The fact that the size of its fruiting structures did not increase proportionately with its increased growth strongly indicated that their diminutive nature constituted an inherent and distinguishing character of this particular slime mold. Nevertheless, there remained the possibility that the smallness of its sorocarps might in some manner be related to and result from some property of the associated bacteria. To test this possibility, and to provide a reliable basis for comparison with other species of the Dictyosteliaceae (Raper, **1937**, **1940a**; Raper & Thom, **1941**) the slime mold was placed in pure-mixed culture with the following selected bacterial species: *Escherichia coli* (Migula) Castellani and Chalmers, *Pseudomonas fluorescens* Migula, *Serratia marcescens* Bizio (*Bacillus prodigiosus* Flügge), and *Bacillus megatherium* DeBary.

The growth of the slime mold was approximately the same in association with the "stock" bacterium, *Bacillus firmus*, and each of the substitute associates upon quarter-strength hay-infusion and 0.05 per cent peptone agars. Upon richer media including full-strength hay-infusion and dung-infusion agars growth was generally more luxuriant with *E. coli* than with either of the other associates. The colonies of this species were entirely consumed as a rule, whereas the colonies of other species commonly were only partially consumed. Very unsatisfactory results were obtained with *Pseudomonas fluorescens* and *Serratia marcescens* upon unbuffered media containing 0.1 per cent or more of peptone. However, by adding adequate phosphate buffers (M/100 in KH_2PO_4 and in $Na_2HPO \cdot 12H_2O$) and subsequently adjusting the medium to pH 5.0 or 5.5 rich cultures were obtained with both associates.

Upon the weaker substrata the majority of sorocarps in every instance were produced singly. Upon more concentrated media, where the growth of the slime mold was much greater and the number of fructifications correspondingly increased, there was a pronounced tendency for the sorocarps to be clustered. In no case, however, were individual fruiting structures materially larger

than in the less luxuriant cultures. It was obvious, therefore, that the small dimensions of its sorocarps represented an inherent character of this slime mold, and was not the result either of the bacterial species with which it was associated or the substratum upon which it was cultivated.

In cultures with *Serratia marcescens* the myxamoebae of *Dictyostelium minutum,* like those of *D. mucoroides* as recently reported by Raper and Thom (**1941**), either digest or discard the red bacterial pigment, *prodigiosin,* during the feeding process. Consequently, the myxamoebae remain practically uncolored and in fruiting build sorocarps characterized by colorless stalks and colorless to milk-white sori. In this, as in a number of other respects, the species closely resembles *D. mucoroides.* There is, in fact, every reason to believe that the two slime molds represent possible extremes in a somewhat graduated series.

SPORES

The spores of *Dictyostelium minutum* are characteristically elliptical (capsule-shaped), hyaline, and in size commonly range between 5 μ–7 μ × 3 μ–3.5 μ. Individual spores may be larger or smaller, and in addition may be either more or less elongate than is indicated by the above figures. Subspherical spores are occasionally seen. In all of these characters the spores resemble closely those of the very common species *D. mucoroides.* In fact, it is apparent from extensive comparative study that less difference in size and shape exists between the spores of *D. minutum* and some typical strains of *D. mucoroides* than between certain strains which unquestionably belong together in the latter species. The striking similarity between the spores of the two slime molds is shown in figure 4.

MYXAMOEBAE

Spore germination in *Dictyostelium minutum* is accomplished, as in other species of the Dictyosteliaceae, by a longitudinal splitting of the spore case followed by an emergence of the spore content as an amoeboid protoplast, or myxamoeba. During the ensuing vegetative period the myxamoebae vary appreciably in size

and differ even more markedly in shape, depending upon whether they are quiescent, feeding, or actively moving.

Quiescent myxamoebae are typically rounded in form and show few or no pseudopodia. Ectoplasmic and endoplasmic zones cannot be distinguished in such individuals normally, nor is a contractile vacuole generally evident. The nucleus may or may not be distinguishable in unstained individuals. The bodies of such rounded myxamoebae range from 8 μ–10 μ in diameter with the majority measuring approximately 9 μ. Individuals, however, not infrequently exceed this range, and often fall below 8 μ in diameter.

When actively feeding upon relatively scattered bacterial cells, the myxamoebae of *Dictyostelium minutum* vary greatly in shape and in apparent dimensions but commonly range from 7 μ–10 μ $\times$ 12 μ–18 μ. Furthermore, their shape is constantly changing as the direction of movement is shifted and as pseudopodia are extended and retracted. Commonly, however, the myxamoebae appear roughly triangular in form, the body being broadened along the advancing front and tapering more or less abruptly toward the area of the contractile vacuole which characteristically occupies a posterior position. In contrast to quiescent individuals, ectoplasmic and endoplasmic areas are clearly distinct. The former usually appears as an irregular, non-granular crescent-like band, extending across the anterior part of the body and thinning rapidly along either side. Extensions of the ectoplasm in the form of pointed pseudopodia are not uncommon and feeding is frequently facilitated by such projections. Usually the entire anterior surface acts as a feeding front and bacteria are ingested simply by the invagination of the body membrane. Whereas bacteria can apparently be ingested at any point on the body surface, feeding occurs primarily along the anterior of the body, possibly because as the myxamoeba advances this area in particular contacts large numbers of bacterial cells. The bulk of the amoeboid body consists of endoplasm which is hyaline and finely granular. This normally contains, however, some coarser granules together with a number of more or less opaque food vacuoles inclosing bacterial cells in various stages of digestion. A single nucleus occupies a position near the center of the body. Commonly two or more contractile

vacuoles are observed in the same individual but they regularly merge before being discharged. The movement of actively feeding myxamoebae is relatively slow and apparently at random for their direction is almost constantly changing. Rapidly moving individuals, on the other hand, are commonly limax-shaped, and a uniform direction of movement may persist for appreciable periods. Feeding by such elongate myxamoebae is much reduced. Going a step further, feeding ceases altogether as the myxamoebae become increasingly elongate, 7 μ–8 μ × 18 μ–24 μ, at the time of initiating the fruiting phase.

The feeding habits of the myxamoebae of *Dictyostelium minutum,* like those of *D. discoideum* (Raper, **1937**), can be studied most satisfactorily by growing the slime mold in association with *Bacillus megatherium.* As in the case of *D. discoideum* the spores of the bacillus are not digested.

PSEUDOPLASMODIUM FORMATION

The specific characters of *Dictyostelium minutum* begin to appear with the initiating of the fruiting phase. Whereas the myxamoebae are seemingly alike in this slime mold and in other species, marked differences in their behavior become obvious in the early stages of aggregation. In thin cultures such as those resulting when the slime mold is cultivated upon weak peptone or dilute hay-infusion agar, the myxamoebae of *D. minutum* normally become oriented toward definite centers of aggregation, but the resulting organizations are regularly much smaller than in other species of the genus, *viz. D. mucoroides, D. purpureum,* and *D. discoideum.* Pseudoplasmodia of this type are shown in figure 1, *E* and *F,* and, except for their diminutive size, suggest the pseudoplasmodia of the larger species. The maximum diameter of these pseudoplasmodia rarely exceeds 1.5 mm. in contrast to 1 cm. or more for the larger species. Furthermore, under conditions most favorable for the development of radiate pseudoplasmodia, many organizations reveal this pattern imperfectly or not at all. Frequently pseudoplasmodia consist of sheet-like masses of myxamoebae flowing towards aggregation centers rather than of definitely converging streams (FIG. 1, *C* and *D*). Less

commonly, aggregates fail to show definite centers and the myxamoebae comprising an apparently single pseudoplasmodium do not show uniform orientation toward any particular point of convergence. This condition is clearly illustrated in figure 1, *G*. In such pseudoplasmodial masses, however, one or more definite centers subsequently appear and toward these the myxamoebae converge.

In dense cultures such as result upon hay-infusion agar when the slime mold is cultivated with *E. coli,* aggregation is not accomplished by the inflowing of even poorly accentuated streams of myxamoebae. It is effected instead by the blocking out of masses of myxamoebae and the subsequent emergence from these of small and, for the most part, typical fruiting structures as shown in figure 2. This is of particular interest, for the phenomenon is essentially comparable to that described by the writer for *D. discoideum* and other large species when these are cultivated upon much richer substrata (**1940b**). In the general pattern of the organizations established, pseudoplasmodium formation in *D. minutum* upon quarter-strength hay-infusion agar is comparable to that of *D. mucoroides* upon full-strength hay-infusion agar; whereas pseudoplasmodium formation in *D. minutum* upon full-strength hay-infusion agar is comparable to that of *D. mucoroides* upon media containing 1.2 per cent each of peptone and some sugar fermentable by the associated bacteria (Raper, **1939**). A possible explanation for this behavior is suggested. The number of *D. minutum* myxamoebae that can effectively coöperate in fruit formation is less than the number than can similarly coöperate in a large species such as *D. mucoroides.* In addition, for each species there exists a definite relationship between the quantity of myxamoebae that can coöperate in fructification and the richness of growth at which pseudoplasmodia of radiate form give way to pseudoplasmodia of block-form. Similar patterns of aggregation thus occur upon relatively poor media in a species characterized by small pseudoplasmodia and upon rich substrata in a species characterized by large pseudoplasmodia. Nevertheless, the general conduct of the two slime molds is basically the same. Apparent differences in their behavior and development during the fruiting process result only

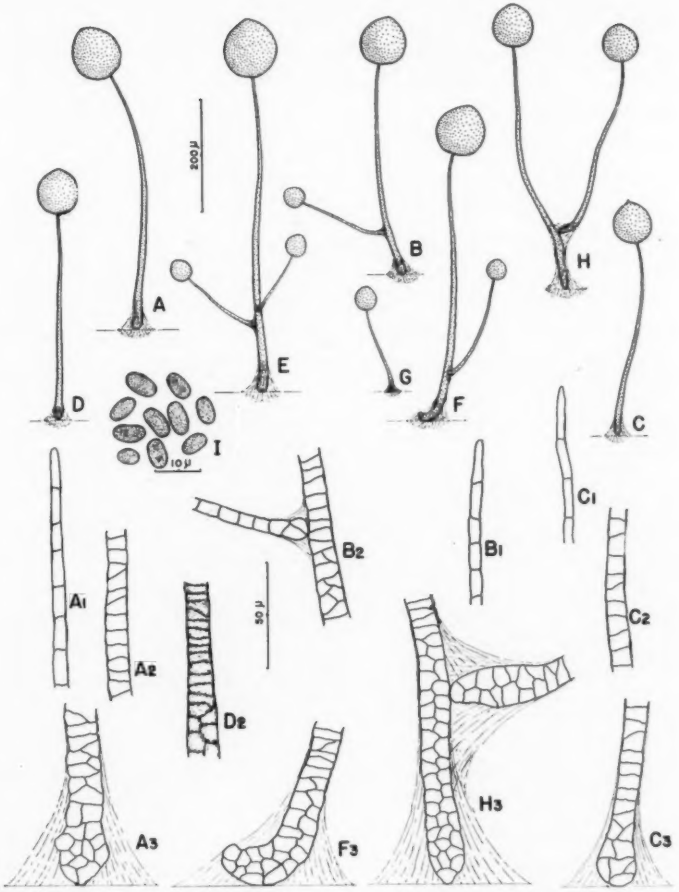

FIG. 3. Mature sorocarps of *Dictyostelium minutum*. *A–G*, camera lucida sketches of typical sorocarps showing relative proportions. A_1, A_2, and A_3, cellular structure of terminal, central, and basal portions of sorocarp *A*. B_1, terminal portion of sorocarp *B*. B_2, structure and anchorage of branch in same sorocarp. C_1, C_2, and C_3, apical, central, and basal portions of sorocarp *C*. D_2, detail of cellular structure near sorophore base, sorocarp *D*. F_3, recurved base of sorocarp *F*. H_3, detail of branch anchorage in sorocarp *H*. *I*, spores. Scale variable as indicated.

from the different quantity of myxamoebae involved in the fruiting organizations of the two species.

The pseudoplasmodia have no migrating stage such as characterizes *Dictyostelium discoideum* (Raper, **1935**). Sorocarps are invariably developed at the sites of pseudoplasmodium formation.

SOROCARPS

The characters which distinguish *Dictyostelium minutum* as a species attain their full expression in the development of the mature fructifications, or sorocarps. Typically, a sorocarp consists of a short, tapering, upright stalk, or sorophore, bearing at its apex a colorless to milk-white spore head, or sorus, of comparatively large size. The sorophore rises vertically into the air and is, as a rule, securely anchored to the substratum by abundant slime, which in effect acts as "guys" to hold the entire body in an upright position (FIG. 3). The sorocarp may be either simple or "branched" (FIG. 3), and fruiting structures of the latter type are much more common in this than in other species of *Dictyostelium*. There is no regularity either in the dimensions or placement of branches. Furthermore, in its pattern and in the manner of its anchorage each individual branch can be considered as an independent sorocarp, usually, but not always, of reduced size (FIG. 3, *H*). Primarily, it differs from a solitary sorocarp in that it derives anchorage and support from another sorocarp rather than directly from the substratum.

Dictyostelium minutum differs from other species of the genus particularly in the dimensions and pattern of its sorophores. Under optimum cultural conditions these are characterized by a marked but gentle taper as illustrated in figure 3. They occasionally attain a millimeter in length but usually fall within the range from 550 μ–850 μ. Smaller structures are not uncommon. In their somewhat swollen basal portions, sorophores are normally 12 μ–20 μ in diameter and commonly two or three cells thick. Stalk bases four cells in thickness are relatively rare but not abnormal. Typically, the cells of the basal area are irregular in shape and parenchyma-like. Just above the basal area the sorophore commonly narrows to 10 μ–15 μ in diameter and is usually

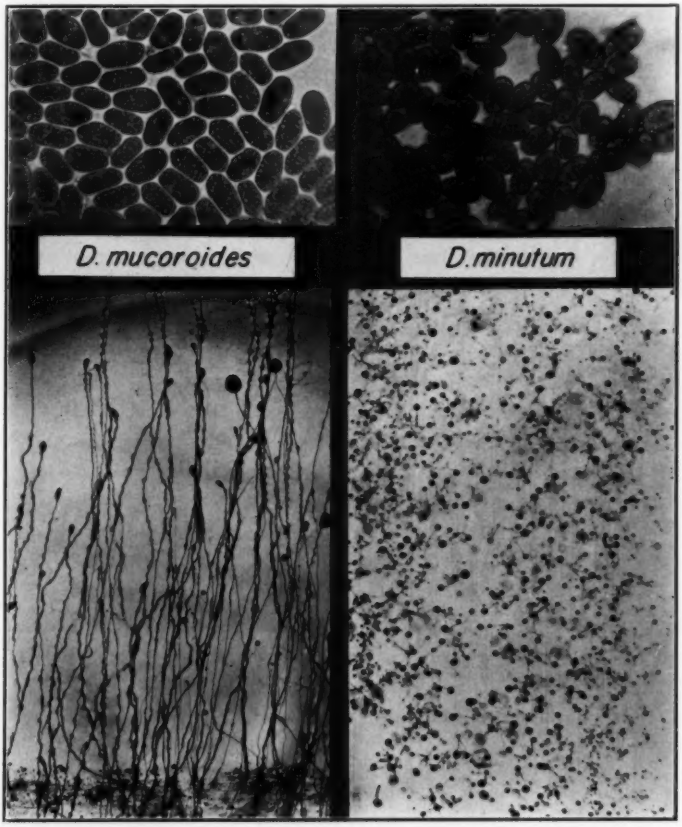

FIG. 4. Comparative size and pattern of spores and sorocarps of *Dictyostelium minutum* and *D. mucoroides*. Cultures grown under identical conditions in association with *Escherichia coli* upon hay-infusion agar in one-sided illumination. Spores of each species × 1000. Sorocarps of *D. mucoroides* × 3.6; sorocarps of *D. minutum* × 7.2.

one or two cells in thickness. When arranged in a single tier the cells of this portion of the stalk are characteristically disk-shaped and horizontally flattened (FIG. 3, D_2). In their central area stalks commonly range from 7 μ–10 μ in diameter and normally consist of a single row of more or less isodiametric cells. In their terminal regions such stalks consist of a tier of vertically

elongate cells, commonly 3 μ–5 μ in diameter and 15 μ–10 μ in length.

Compared with the more common species of the Dictyosteliaceae, the sorocarps of *Dictyostelium minutum* are extremely delicate (FIG. 4). Nevertheless, they are regularly characterized by comparatively large sori that commonly range between 75 μ–125 μ in diameter and may occasionally reach 150 μ. The brevity and thinness of the sorophores, as noted above, coupled with the relatively large dimensions of the spore masses lend to the sorocarps of this slime mold a striking and distinctive pattern (FIG. 1, *H* and *I*). Once its sorocarps have been observed in culture the slime mold is unmistakably distinguishable from the more common species of the genus.

As in other members of the group, the sorus consists of a large number of spores suspended in a small droplet of fluid expressed from the myxamoebae during their maturation into spores. The whole mass hangs from the apex of the sorophore. The manner in which it is borne accounts for the somewhat tear-drop shape of the sorus.

The above description is based primarily upon solitary sorocarps characteristic of thin cultures, but it likewise covers adequately stalk and sorus patterns of clustered sorocarps. In the writer's experience, under optimum cultural conditions, clustered sorocarps differ from solitary structures primarily in being more frequently branched.

DISCUSSION

Two small species of *Dictyostelium* characterized by white spore heads have been previously reported, namely: *D. lacteum* van Tieghem (**1880**) and *D. brevicaule* Olive (**1901**). The description of neither species adequately pictures the slime mold under consideration. *Dictyostelium lacteum* was depicted as possessing stalks consisting of a single tier of cells, a character that is strongly suggestive of *D. minutum*. However, sorocarp dimensions were not given, and its spores were reported as spherical and very small, 2 μ–3 μ in diameter. Myxamoebae giving rise to such small spores would undoubtedly differ markedly from those of *D. minutum*. *Dictyostelium brevicaule* was described as characterized by

short and rather heavy stalks bearing comparatively large sori. The latter character is remindful of *D. minutum,* but the stalks of this slime mold when grown under favorable culture conditions are anything but rigid. In addition, the sorocarps of *D. brevicaule* were described as ranging from 1–3 mm. high and therefore definitely larger than those of *D. minutum.* In the writer's experience strains possessing the characters of *D. brevicaule* have been occasionally isolated. While there is some question whether this represents a separate species or a short stalked variation of *D. mucoroides,* there is no question but that it is definitely different from *D. minutum.*

In the size and pattern of its spores and in the appearance and behavior of its vegetative myxamoebae, *Dictyostelium minutum* bears a striking resemblance to the exceptionally common species, *D. mucoroides.* The fruiting structures of the two slime molds, however, are markedly different, and it is this difference which sets them apart as distinct species. It is important to note that the distinguishing characters in this case arise not from apparent morphological or physiological differences between the myxamoebae which constitute the basic units in the two organisms, but rather from the way in which these units organize and differentiate to effect fructification. The limited number of myxamoebae that can effectively coöperate in building a sorocarp of *D. minutum* is an inherent and specific character of the species. Likewise inherent and specific is the particular way in which the myxamoebae differentiate to produce a diminutive fruiting structure characterized by a short, thin tapering stalk supporting a comparatively large spore head.

In *Dictyostelium minutum,* as in *D. mucoroides* and *D. purpureum,* sorocarp formation is normally initiated by the time the aggregation of myxamoebae comprising the pseudoplasmodium is completed. Unlike these species, however, *D. minutum* invariably builds its sorocarps directly above the sites of myxamoebic aggregation. In side-illumined cultures of the larger species one commonly finds a sorus borne as much as 5 cm. from the center of pseudoplasmodium formation, the intervening distance being bridged by a continuous and for the most part horizontal stalk of fairly even diameter. In *D. minutum* there is no comparable

light response. In fact, in repeated experiments the writer has been unable to detect any consistent response to light. Two possible explanations of this behavior present themselves. The pseudoplasmodium and young sorocarp of this species, unlike other members of the Dictyosteliaceae, may be basically insensitive to light. On the other hand, the young sorocarp may be of such small dimensions, or the stage of functional differentiation may be so advanced at the close of aggregation, that light sensitiveness, though present, cannot gain expression. While *D. mucoroides* and *D. purpureum* are generally considered to be extremely sensitive to light (FIG. 4), developing sorocarps less than 5 or 6 mm. in length do not normally show a marked phototropic response. In view of this behavior the absence of an evident phototropic response is not surprising when it is recalled that the sorocarp of *D. minutum* rarely exceeds a millimeter in height.

SUMMARY

A new species of *Dictyostelium* isolated from forest litter is described. Because of the diminutive character of its sorocarps the name *D. minutum* is proposed.

In the size and pattern of its spores and in the behavior of its vegetative myxamoebae in culture, the species closely resembles *D. mucoroides*. It is distinguished from this species primarily by the conspicuously smaller dimensions of its pseudoplasmodia and sorocarps and secondarily by the pattern of its completed fructifications. In thin cultures under optimum environmental conditions, radiate pseudoplasmodia appear, but they are not formed as consistently as in the larger species. Mature sorocarps are diminutive and unsubstantial. Typically they consist of short, thin, tapering sorophores bearing comparatively large, colorless to milk-white sori. Normally the sorophore consists of a single tier of cells throughout the greater portion of its length, the individual cells varying in shape from flattened and discoid toward the base to elongate and tube-like at the apex. Branched sorocarps are more common than in any other species of *Dictyostelium*.

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DISCHARGE OF THE SPORANGIOLES OF BIRD'S NEST FUNGI

B. O. Dodge

(with 2 figures)

In a recent number of Mycologia[1] W. H. Diehl has discussed briefly the evidence in support of the idea that the fruiting bodies of *Leptostroma Camelliae,* reported by Zenker on the underside of leaves of *Camellia,* are, in fact, the sporangioles or peridioles of some member of the *Cyathus* group. The attachment of the sporangioles to the leaves as figured by Diehl is clearly not the only way they may be attached. Mr. John J. Shea several years ago called my attention to the fact that the sporangioles of species of *Cyathus* often hang suspended to leaves by the funicular threads, which may vary in length up to two or three inches. I recently received a letter from this gentleman in which he says that for twelve years or more he has made a study of species of *Cyathus* and related genera and has seen many times the sporangioles of four different species attached to leaves by long thin threads or funiculi which he referred to as the "web." He points out that the fungus shoots the sporangioles into the air and that they can be found attached to leaves, twigs and limbs of willows, scrub oaks, or whatever plants are growing near the developing cups or basidiocarps. As to the distance the sporangioles are shot, he says: "I find some as high as 13 feet, some 10 feet and all the way down to a few inches from the ground. By actual measurement I found some attached to leaves of a scrub oak which were 13 feet above the ground." He says he grew the plants in the house in old cheese boxes and he discovered that the sporangioles were shot onto the window panes as well as up under the awning. He tells me personally that they are shot out at night and not when the peridia are wet, but some time afterwards. He has recently shown me that the sporangioles of a species of *Crucibulum,* which

[1] Mycologia **33**: 215–219. 1941.

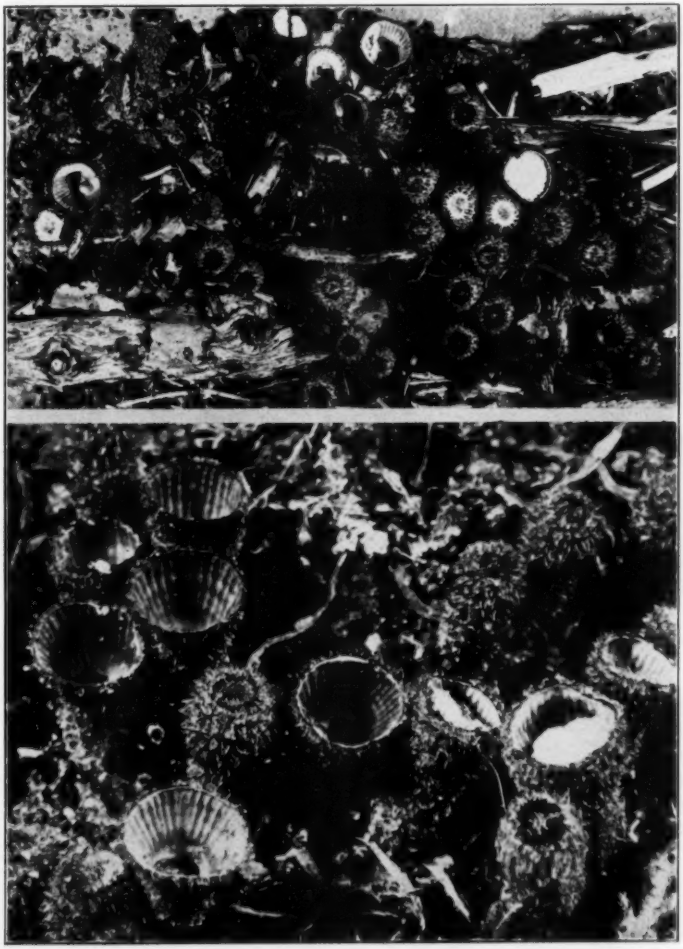

FIG. 1. Photographs of specimens of *Cyathus striatus*, located for the writer by Dr. F. J. Seaver. This group of basidiocarps was growing on decaying sticks and leaf mold, others were growing directly on coal ashes in the dump. Above, the mouths of most of the peridia are still closed. The snow-white epiphragm is clearly shown in several cases. Below, peridia are fully open.

has white peridioles and yellowish buff cups, may be found attached directly to the leaves and not hanging in the air. Some of them may be found on the lower side of the leaf, others on the upper side. As a rule, if one examines these attached sporangioles he finds a sort of a net or network attached to a short thread, but the morphological features here are quite different from those characteristic of species of *Cyathus,* which have a well organized funiculus from which a very long thread is spun out. When Mr. Shea first called my attention to this phenomenon of sporangiole discharge several years ago, I asked him to get in touch with A. H. R. Buller, which he said he did by letter. Buller's reply, Shea said, was rather noncommittal, at least he was not aware that the periodioles were discharged in this manner. Recently I have had the occasion to examine rather superficially the mode of attachment of the sporangioles of *Cyathus striatus* to the peridium. There is at the base of the funiculus a volva-like cup which is sometimes funnel-shaped and at others more or less cylindrical, composed of a web-like growth. This is attached firmly to the inside of the peridium. The peridiole with its rather short bulbous stalk resembles in miniature a sporophore of a *Boletus.* The bulbous base of the funiculus is attached by a thread to the volva sac either at a lower part or sometimes rather near the margin on the inside of the cup, in which case many very fine attachment threads are involved.

If one slits open the peridium and grasps a peridiole with a pair of forceps, holding the peridium with the other hand, he can see under a binocular dissecting microscope how the funicular thread is spun out as it unwinds and spins up and down and from side to side in the funicular sac, which is consumed during the process of spinning and which usually finally disappears, becoming a part of the thread itself. Depending somewhat on the state of maturity and the moisture of the cup and the way one handles the setup, the spinning process may begin either directly under the peridiole or down near the lower end of the funiculus. This is best observed when no free water is present. One is reminded, when he sees how the funicular thread can be pulled out rapidly without a break, of the way parachutes must be carefully packed or the life

saver's lines must be coiled, to insure against tangling at the critical moment.

The Tulasnes [2] long ago described and figured very beautifully this phenomenon of spinning out of the funiculus. They also figured rather accurately the various structures involved. They did not, however, observe that the peridioles were discharged with violence, neither did Sachs,[3] who studied *Crucibulum*. The Tulasnes evidently thought that the funicular thread was enclosed in

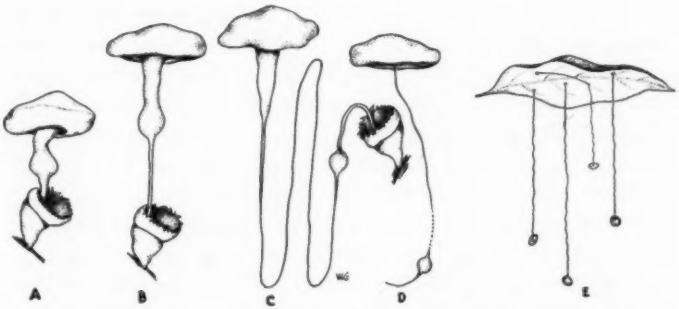

FIG. 2. Sketches showing (*a*) a lens-shaped peridiole with its stalk or funiculus having a bulbous end which is attached to the inside of the "volva"; (*b*) the attachment or anchor thread has been stretched out by pulling on the peridiole with a forceps; (*c, d*) the funicular thread has been spun out its entire length, which finally becomes three or more inches long before it breaks away. It may break directly at the bulbous end, or the anchor thread attaching the bulb to the "volva" may break. If the moisture content is just right, the thread begins to spin from directly beneath the peridiole; (*e*) sketch of a leaf showing three or four peridioles hanging by threads of different lengths.

a sac which was a distinct structure. The funicular thread, although very fine, is comparatively strong when dry. Even when moist it can hold a weight much more than the weight of a basidiocarp or peridium. It is difficult to imagine just how a pressure can be built up which will be sufficient to throw the peridioles 10 or 15 feet into the air. One finds these structures of *Crucibulum* attached to the upper side of the leaves as well as the lower side,

[2] Tulasne, L. & Tulasne, C. Ann. Sci. Nat. III. 1: 41–107. *pl. 3–8.* 1844.
[3] Bot. Zeit. 13: 849. 1855.

showing that they must have been originally shot higher than the leaves themselves in order to fall on the upper surface. The whitish peridioles of a *Crucibulum* are usually attached to the leaf by the lower surface of the peridiole and not infrequently a short white thread extends out from the peridiole along the leaf to which it sticks. There is frequently a web-like growth attached to the thread. No doubt those peridioles which are provided with a definite funicular structure often hang down from leaves by the thread which may shorten or lengthen depending on the moisture.

We placed the specimens shown in the photograph under a bushy *Amelanchier,* early in August. By September 1st there were quite a number of the peridioles hanging from the under side of the leaves, the threads varying from one-half to three inches in length. The short threads represent cases where the greater length of the thread is stuck to the leaf, only a short part being free. Several peridioles were attached directly to the under side of the main stems which stand at about a forty-five degree angle. The peridioles themselves are loose, however, and not stuck to the bark as would first appear. The thread can be seen stuck to the bark for its entire length. Wetting the bark often releases the thread sometimes only for a part of its length so that the peridioles hang down normally.

THE NEW YORK BOTANICAL GARDEN

SOME LEAF SPOT FUNGI ON WESTERN GRAMINEAE [1]

RODERICK SPRAGUE [2]

(WITH 1 FIGURE)

The fungi discussed in this article represent incompletely known or undescribed species that may be encountered by workers dealing with diseases of western range and pasture grasses. The collections that are mentioned in this note are filed either at Oregon State College (O.S.C. numbers) or are in the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture. The fungi will be discussed individually as follows:

Phyllosticta Owensii sp. nov.

Maculis elongatis, griseis; pycnidiis sparsis, globosis, erumpenti-superficialibus, nigris, tarde ostiolatis, 70–100 μ, pycnosporulis numerosis, bacillaribus aseptatis, hyalinis 2.3–4.6 $\times$ 1.0–1.4 μ.

Hab. in foliis vivis et emortuis *Dactylidis glomeratae*, Waldport, Oregon. Sept. 24, 1939.

Lesions elongate, gray, pycnidia sparse, globose, superficially erumpent, black, tardily ostiolate, 70–100 μ; spores numerous, bacillar, non-septate, hyaline, 2.3–4.6 $\times$ 1.0–1.4 μ.

On living, dead or salt-spray-injured leaves of *Dactylis glomerata* across the Alsea River from Bailey's Landing, 3 miles east of Waldport, Oreg., and at Yaquina John Point, Waldport, Oreg. (Type, O.S.C. *8,000*). Associated with *Scolecotrichum graminis* Fuckel.

[1] Coöperative investigations between the Division of Forage Crops and Diseases and Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon and North Dakota Agricultural Experiment Stations. Approved by the Director of the Oregon Agricultural Experiment Station for publication as Technical Paper 372, contribution from the Department of Botany.

[2] Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The writer is indebted to A. G. Johnson and associates for aid in the preparation of this paper, and to Miss Edith Cash for checking the Latin descriptions.

This fungus has some resemblance to *Phyllosticta striolata* Sacc. (**11**, p. 45) on *Brachypodium distachyon* but differs from it in that the ostioles are smaller or sometimes absent and the spores and pycnidia are larger. The spores of *Phyllosticta Dactylidis* Gonz. Frag. (**1**) are not bacillar as in *Phyllosticta Owensii* but are ovoid to oblong, 3–5 × 2–3 μ and are therefore twice as broad as the latter.

Phyllosticta Owensii is placed in *Phyllosticta* instead of *Phoma* not because it occurs on leaves (which is of no importance in itself) but because there seems to be considerable doubt as to the actual authenticity of the genus *Phoma*. *P. Owensii* has more of the appearance of the so-called *Phoma* group than of *Phyllosticta*. *Phyllosticta* is usually considered as having relatively thin-walled pycnidia with dark pigment only in the cells adjacent to the ostiole, while *Phoma* is typically recognized as having compact dark pycnidia, with strongly carbonized or colored walls, accompanied by stout ramifying mycelium in the substratum. Many of the *Phoma* species occur on woody parts, often as saprophytes (**3**) and this in itself induces dematiaceous tendencies in the pycnidial development. The common leaf spotting Phyllostictae on their more succulent substrata develop a paler, thinner-walled fruiting body and actually are fundamentally no different from their darker associates on woody or silicified host parts except as chance has given them different environments. *Phyllosticta Owensii* is weakly parasitic on leaves, and shows decidedly "*Phoma*-like" pycnidia, more so than the other species of *Phyllosticta* described or discussed in this article. In assigning this species to *Phyllosticta* we note that Grove (**3**) disparages those that use *Phoma* as a "dumping-ground" for ill-understood fungi. The same can be said for *Phyllosticta,* as continued study will no doubt show that many of these fungi have septate spores. Until the happy day when everything is known about all fungi, it seems necessary to assign these fungi with one-celled spores, borne in ostiolate pycnidia, to some genus and species. Except for a few overworked ones (cfr.: *P. sorghina*) there are relatively few species of *Phyllosticta* on Gramineae. It appears to be necessary, therefore, to describe three species of *Phyllosticta* in this article, including *P. Owensii* above noted.

Phyllosticta anthoxella sp. nov.

Pycnidiis erumpentibus, nigris, globosis, 35–50 μ, ostiolatis (4–5 × 10–12 μ), pycnosporulis bacillaribus, aseptatis, hyalinis, guttulatis 5–9 × 1–1.6 μ. Hab. in foliis dejectis *Anthoxanthi odorati*. Oregon.

Pycnidia scattered but tend to be in rows parallel to the leaf veins, erumpent, black, minute, 35–50 μ, ostiolate (4–5 × 10–12 μ)

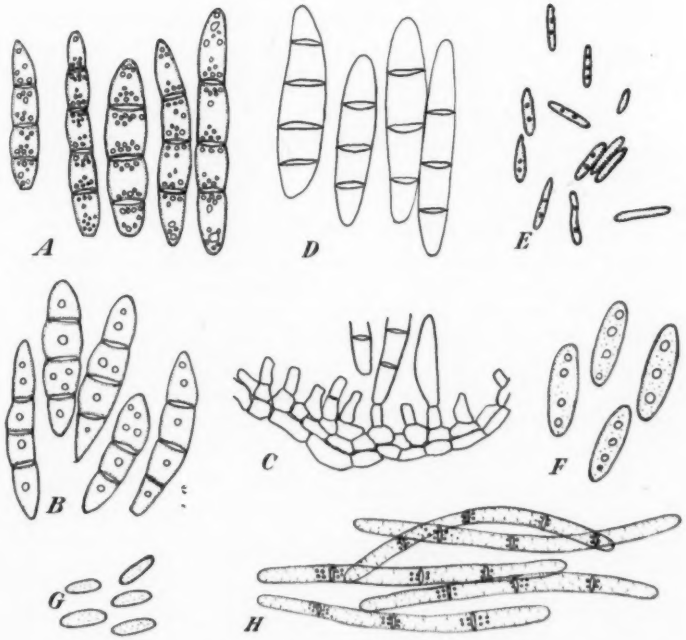

FIG. 1, *A*, pycnospores of *Stagonospora subseriata* (Desm.) Sacc. var. *maculata* Grove on *Dactylis glomerata*, State College, Pa., collected by S. J. P. Chilton; *B*, pycnospores of *Stagonospora subseriata* on *Elymus mollis*, Manhattan, Oreg., Aug. 16, 1939; *C*, cross section of pycnidial wall of *Stagonospora subseriata* var. *maculata* from State College, Pa.; *D*, pycnospores of *Stagonospora subseriata* on *Deschampsia caespitosa*, Lyndhurst, Hampshire, Engl., collected by Cooke; *E*, pycnospores of *Phyllosticta anthoxella* Spr. from type collection; *F*, pycnospores of *Phyllosticta Roglerii* Spr. from type collection; *G*, pycnospores of *Phyllosticta sorghina* Sacc. from *Setaria viridis*, Langdon, N. Dak., Aug. 2, 1940; and *H*, pycnospores of *Septoria Secalis* Prill. & Del. var. *Stipae* Spr. on *Stipa viridula*, from type collection. (All drawings made with the aid of the camera lucida. Magnification × 1,000.)

surrounded by darker, sepia-colored cells, pycnospores exuded in long chains in water mounts, hyaline, aseptate with 2 to several small guttulae, cylindrical or bacillar, 5–9 × 1–1.6 μ.

On dead basal leaves of *Anthoxanthum odoratum*, associated with *Colletotrichum graminicolum* and *Titae* sp. North shore of Triangle Lake, along highway, Lane county, Oreg., May 10, 1938 (Type, O.S.C. *264*).

There are no fungi on *Anthoxanthum* comparable to this except possibly *Septoria anthoxina* Gonz. Frag., which has considerably larger spores (25–30 × 1.2–1.5 μ), and the few species of *Phyllosticta* that approach *P. anthoxella* in spore dimensions. These latter, however, have much larger pycnidia. The spores of *P. anthoxella* are illustrated in figure 1, E.

Phyllosticta Roglerii sp. nov.

Maculis parvis, elongatis, albidis; pycnidiis conspersis flavo-brunneis, globosis, erumpentibus, ostiolatis (6–12 μ), parenchymaticis, 50–100 μ diam.; pycnosporulis ellipsoidis, aseptatis, hyalinis, guttulatis, 11–15 × 2.5–4.5 μ.

Hab. in foliis vivis *Digitariae sanguinalis*. Iowa.

Spots small, elongate, white, pycnidia scattered, yellow brown, globose, strongly erumpent, thin walled, composed of polyhedral cells, ostiolate (6–12 μ) 50–100 μ; pycnospores ellipsoid, non-septate hyaline, 3–6 guttulate, 11–15 × 2.5–4.5 μ (FIG. 1, *F*).

In living leaves of *Digitaria sanguinalis* (L.) Scop. associated with *Colletotrichum graminicolum* in fields near Albia, Iowa. Sept. 10, 1940. Collected by George Rogler and R. Sprague.

It is suspected that this species is a developing phase of a larger fungus.

PHYLLOSTICTA SORGHINA SACC. ON SETARIA VIRIDIS

The common weed and pasture grass *Setaria viridis* is affected with an eye spot type of leaf injury in the vicinity of Langdon, North Dakota. The lesions are irregular, mostly sub-circular, 3–8 mm. diameter, pale straw to isabelline with narrow purple borders. The pycnidia are small, 60–100 μ, black, sub-globose, fuligenous walled, strongly erumpent, but not mammiform. The spores are non-septate, hyaline, eguttulate, and exude in cirri when the pycnidia are placed in water. The spores are ellipsoid with rounded ends 4.5–6.5 × 2.1–2.5 μ (FIG. 1, *G*).

TABLE 1

COMPARISON OF *Phyllosticta sorghina* WITH RELATED SPECIES

Fungus	Host	Country	Pycnidia	Pycnospores
			μ	μ
P. sorghina Sacc. (**9**)	*Sorghum vulgare*	Italy	—	5×2
P. Sacchari Speg. (**12**)	*Saccharum officinarum*	Argentina	90–100	4–6×2–3
P. Setariae Ferr. (**2**)	*Setaria lutescens* (*S. glauca*)	Italy	110–120	6.5–7×2.5–3
P. glumarum-Sorghi P. Henn (**4**)	*Sorghum vulgare*	Congo	40–60	4–5×2–3
P. glumarum-Setariae P. Henn (**4**)	*Setaria lutescens* (*S. aurea*)	Congo	60–70	3.5–4×2–2.5
P. phari Speg. (**13**)	*Pharus glaber*	Argentina	50–75	4–5×2
P. Penicillariae Speg. (**14**)	*Pennisetum glaucum* (*Penicillaria typhoidea*)	Senegal	120–150	4–5×2
P. sorghina Sacc.	*Setaria viridis*	United States	60–100	4.5–6.5×2.1–2.5
Phoma insidiosa Tassi	*Sorghum vulgare*	Abyssinia	70–80	6×2–2.5

This fungus differs from the description of *Phyllosticta sorghina* Sacc. in not having 2-guttulate spores and is not associated with an *Ascochyta*, although it could and may be a stage of this genus. Otherwise the fungus on *Setaria viridis* from Langdon is the same as *P. sorghina* Sacc. and it is assigned to this species.

In this connection, a number of related species were compared particularly for range of sizes of pycnidia and pycnospores. This comparison is given in table 1. While there are some minor differences, yet these are not considered significant as far as species delimitation is concerned, and accordingly these species names are brought together under one species name, *Phyllosticta sorghina* Sacc. being the oldest, and the following synonymy is proposed:

PHYLLOSTICTA SORGHINA Sacc.	1878	(**9**, p. 140)
SYN:		
P. Sacchari Speg.	1896	(**12**, p. 239)
P. Setariae Ferr.	1902	(**2**, p. 18)
P. glumarum-Sorghi P. Henn.	1907	(**4**, p. 101)
P. glumarum-Setariae P. Henn.	1907	(**4**, p. 101)
P. phari Speg.	1910	(**13**, p. 337)
P. Penicillariae Speg.	1914	(**14**, p. 129)
Phoma insidiosa Tassi	1898	(**18**, p. 8)

Some of these, particularly *Phyllosticta Penicillariae, P. glumarum-Sorghi, P. Sacchari,* and *P. glumarum-Setariae,* sometimes at least, have lenticular rather than globose pycnidia. Otherwise they appear to belong to one species.

"*Phyllosticta Sorghi* Anzi," referred to by Saccardo (**9**) in describing *P. sorghina,* is an older name than *P. sorghina* but it was not validly established because no pycnidia nor spores were described.[8]

The name "*Phyllosticta Sorghi* n. sp." was originally applied by Rabenhorst in 1876 (**7**, No. *2162*) to specimens showing characteristic leaf spots on *Sorghum saccharatum* collected by M. Anzi in northern Italy. With the exsiccati specimen, Rabenhorst gave no description either of the spots or of any fungus. In 1876, Raben-

[8] The discussion of *Phyllosticta Sorghi* Rab. is by A. G. Johnson who very kindly traced material and literature available in the Bureau of Plant Industry, Washington, D. C.

horst (**8**, p. 120), in commenting on the exsiccati specimen, referred to the name, properly, as "*Ph. Sorghi* Rabh." and noted that no description was given. In 1878, Saccardo (**9**), in describing *P. sorghina,* referred to the name as follows: "*Phyll. Sorghi* Anzi in Rabh. F. E. n. 2162 sistet forman leptostromaceam v. phyllachoraceam, cujus sporas proprias non vidi." In 1883, Thümen (**19**, No. *2196*) distributed more of apparently the same material collected by M. Anzi. His label reads as follows: "2196. *Phyllosticta Sorghi* Anzi in Rabh. Fung. europ. No. *2162*. Lombardia: Como in foliis vivis *Sorghi saccharati* Pers. Com. Dr. G. Winter. Leg. M. Anzi." The material is identical with that of the Rabenhorst No. *2162*, and likewise shows neither pycnidia nor spores. Both Saccardo and Thümen erred in ascribing the species name to Anzi, who was but the collector. As pointed out by Rabenhorst (**8**), the name was first applied in print by him, and if referred to, should be written: *P. Sorghi* Rabh.

In 1884, Saccardo (**10**, p. 65) transferred the still unestablished species name "*P. Sorghi*" to the genus *Depazea*, making the combination "*Depazea* (*Phyllosticta*) *Sorghi* Anzi," denoting that no spores had been found. Here Saccardo referred to "Hedwigia 1876, p. 120" and to "F. eurp. n. 2162" in neither of which Anzi is given as the authority for this unestablished species name. Here also Saccardo described the spots more adequately and added another host, namely, *Sorghum vulgare*, and added: "peritheciis —nullis visis." Thus the name was still a *nomen nudum* as there was no description of the fungus.

Oudemans (**5**, p. 709; 712) lists "*Phyllosticta Sorghi* Anzi" as a synonym for *Depazea Sorghi* Sacc. (Syll. Fung. **3**: 65) on *Sorghum saccharatum* and *S. vulgare*, also without any description of the fungus. Thus he wrongly cites Saccardo and Anzi as the authorities for the species name.

It seems clear, therefore, that the species name of *Phyllosticta Sorghi* Rabh. is non-available as it has never, so far as known, been validly established.

The symptoms on *Setaria viridis* as they occur at Langdon are confusable in the field with common bacterial spots on millets and species of *Sorghum*.

PHYLLOSTICTA SORGHINA SACC. ON TRICHOLAENA ROSEA NEES

Fragmentary material collected on *Tricholaena rosea* (Natal grass) at Pullman, Wash., in 1940, was sent to the writer by George W. Fischer. This material has spores identical in size (5–6.5 × 2.1–2.7 μ) with those on *Setaria viridis* as above discussed. The light colored spots with narrow red borders appear typical for the species. The pycnidia were brown, sub-superficial, ostiolate, globose, 50–90 μ in diameter. Since this grass is closely related to *Setaria*, it appears reasonable to assume that infection on this exotic host probably developed as a result of association with local *Setaria viridis*.

STAGONOSPORA SUBSERIATA (Desm.) Sacc.

This species which was first described on *Enodium coeruleum* has broadly fusiform, mostly triseptate spores, which are constricted or sometimes not constricted at the septa. The type has spores 38–40 × 7 μ. A collection on *Elymus mollis* from Manhattan, Oreg., has spores somewhat smaller, 20–33 × 4.5–5 μ (FIG. 1, *B*), but they are apparently immature ones of *S. subseriata* (compare with FIG. 1, *D*). The almost boat-shaped spores are quite distinct from *Stagonospora arenaria* Sacc., which also occurs on *Elymus* in the region (**16**). Similar material of *S. subseriata* was collected on *Elymus mollis* at Yaquina John Point, Waldport, Oreg., Sept. 24, 1939 (O.S.C. *732*).

Grove has proposed *Stagonospora subseriata* var. *maculata* on *Dactylis glomerata* (**3**), which has the pycnidia arranged in lines in blackish-brown blotches. Specimens of what seems to be this fungus on *D. glomerata* from State College, Pa., sent by S. J. P. Chilton, show similar symptoms and spores which are fusiform, 3–4 septate, yellow with coarse contents (FIG. 1, *A*), and 27–40 × 4.8–6.5 μ, mean size 33 × 5.5 μ. They are borne in globose golden brown pycnidia with walls about 5 μ thick (FIG. 1, *C*). The cylindrical pycnophores are 4–6 × 1.8–2.2 μ. The writer was of the opinion at first that this fungus must be a stage of *Stagonospora arenaria* because of similar symptoms and because *Dactylis glomerata* is a host for *S. arenaria* in Oregon (**16**) and Ohio. However, because of the decidedly different spores of the Penn-

sylvania material, there is no close genetic relation between the two. The writer therefore concludes that Chilton's material is the same as that described by Grove. Further study with more material of *S. subseriata* proper will be needed to determine finally if the definitely parasitic variety on *Dactylis* belongs with the weakly parasitic species proper. Along this line Gonzalez Fragoso (**1**) considers *S. subseriata* to be a phase of *Hendersonia culmicola*. Petrak (**6**, p. 383) lists a fungus on culms of *Dactylis glomerata* as *S. subseriata* (Desm.) Sacc. and states that the spores are 23–44 × 3–6 μ. Finally it should be noted that some collections of the boat-shaped-spored *S. subseriata* show little or no constriction at the septa (FIG. 1, *D*) while others show definite constrictions (FIG. 1, *B*). This is partly explained by the condition of the collections and the age when collected and probably does not represent particularly wide variations.

ROBILLARDA AGROSTIDIS Spr.

Following heavy rains, saprophytic material of *Robillarda Agrostidis* on *Buchlöe dactyloides* (Nutt.) Englm. was collected at Fargo, N. Dak., Sept. 5, 1940. The spores with their tri-furcate appendages were 17–22 × 1.9–2.3 μ, only slightly narrower than the winter material originally described on *Agrostis tenuis* from Oregon (**15**).

CONIOTHYRIUM PSAMMAE Oud.

Coniothyrium Psammae Oud. produces large, elliptical, light buff lesions with prominent vinaceous borders on living leaves of *Calamagrostis nutkaensis* (Presl) Steud. along the coast of Oregon. The few black pycnidia contain hyaline, later brown, elliptical spores 10–13 × 3–4 μ. They are produced by budding from the walls of the unilocular pycnidia on pycnophores, which vary from mere papillae to ones 6 μ or rarely 10–12 μ long. The spores have a slight hilum near one end and contain large, curved, central bodies or nuclei.

On the basis of size and morphology of spores, this fungus belongs in *Coniothyrium* rather than *Sphaeropsis*. The earliest recognizable name appears to be *Coniothyrium Psammae* Oud.

It might be added that material of *Coniothyrium Psammae* collected near Taft, Oreg., on March 25, 1933, had a species of *Mycosphaerella* associated with it (O.S.C. *10,817*) although other material from Waldport, Oreg., collected March 22, 1937 (O.S.C. *749*) did not show any *Mycosphaerella*. The asci from the Taft collection were cylindrical, 50 × 11–13 μ and contained immature spores 17–20 × 5–6 μ.

Septoria Secalis Prill. & Del. var. Stipae var. nov.

This fungus causes a white leaf spot on *Stipa viridula* Trin. in southeastern South Dakota. The material collected at Vermillion in mid-September, after heavy rains, gave indications that the fungus was a very active parasite under such moist conditions. Comparison with all available fungi indicates also that this collection is referable to a variety of *Septoria Secalis* Prill. & Del. in that symptoms are very similar to and the spores are the same general shape as those on rye. This variety described on *Stipa viridula* differs in having longer spores (those on rye are 25–50 × 2.5–3.5 μ), somewhat more prominent pycnidia, and in having spores with less homogeneous contents. The latter character may not be of any importance as the Vermillion material may not be entirely mature, as indicated by the glutinous nature of the oozing cirri.

The description is as follows:

Lesions at first dark brown, circular, soon pale buff in center, finally white to gray-white and elongate; pycnidia prominent, black, globose, 130–190 μ, ostiolate, erumpent; pycnospores sometimes straight but usually slightly and stiffly curved, broadly filiform with rounded and only slightly tapering ends, 3-septate with 2, 4, or several small oil drops on either side of the cross walls, rarely slightly constricted at the septa, 37–54 × 2.6–3.1 μ, oozing out from the pycnidia in large cirri which retain their identity for some time in water (FIG. 1, *H*).

On leaves, culms and sheaths of *Stipa viridula* Trin. at the C.C.C. Camp and Soil Conservation Service Nursery at Vermillion, S. Dak., Sept. 14, 1940. Collected by Olaf Aamodt, Roderick Sprague, and George Rogler (B.P.I. *80,012*).

This fungus is very distinct from *Septoria stipina* Died., which the writer reported elsewhere on *Stipa columbiana* var. *Nelsonii* from a collection made by W. E. Lawrence in the Blue Mountains in Oregon (**17**). *Septoria stipina* from Oregon has very narrow spores, 39–63 × 0.8–1.1 μ.

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NOTES AND BRIEF ARTICLES

The Devil and the Deep

In my work with the polypores, when I tried to break up a large homogeneous group into convenient pieces there was a terrible howl about the lack of generic characters, which I must admit was partly justified. In the boletes I used the color of the spores to segregate genera, as was commonly done with other fleshy fungi. Now this treatment draws heavy fire because *no one had thought of it before,* and also because I did not split the eighty species into ten genera of exactly eight species each! Sorry, but there seems to be no pleasing all the people all the time.—W. A. Murrill.

Correction—The host of the *Septoria,* described in Mycologia **33**: 362. 1941, is, *fide* Dr. A. J. Sharp, not *Tussilago* but *Cacalia atriplicifolia.* This material agrees exactly with what was collected by Demetrio in Missouri and issued as *Septoria Cacaliae* Ellis & Kellerm. by Kabat and Bubak in their Fungi Exsiccati, No. *417.*—John Dearness.

Lepiota Morgani, an Unwholesome Fungus

A case of toadstool poisoning has come to our attention which occurred in Riverside, California, in 1936. About two pounds of an agaric were gathered in the lawn and were eaten by four adults. Four hours after the meal symptoms were noted and were severe for two hours with traces for 24 hours. Symptoms consisted of nausea, cramps, and diarrhea. Two of the persons (women) who ate the mushrooms were severely affected; one man was mildly affected, and one man not affected.

The mushrooms were prepared by sauteing in butter. The specimens used were not seen by a mycologist but other specimens recognized by the users as identical were brought to the Citrus Experiment Station and were clearly *L. Morgani* (Peck) Sacc.

Lepiota Morgani occurs frequently in lawns in Riverside during warm weather and is of general distribution in southern California.[1] It evidently should be avoided as food.—WM. T. HORNE AND IRA J. CONDIT.

MONOGRAPHS OF THE PYRENOMYCETES

A sequel to Wehmeyer's monograph of *Diaporthe* and its segregates has recently appeared in the University of Michigan Studies, Science Series, volume 14. The latest monograph is devoted to the genera *Melanconis, Pseudovalsa, Prosthecium* and *Titania,* and their allies.

In his introduction the author comments on the duel purposes of a classification, i.e. to show natural relationship and to offer a convenient arrangement, and stated that often the latter is overlooked although in his opinion it is often the more important of the two. He explains that in this group it is often impractical to use conidial stages as a bases for segregating genera, because of the obscurity of this stage. If it were so used we still have a large number of forms to deal with in which the conidial stage is unknown, and an attempt to so use it would only add to the confusion already existing.

The monograph, consisting of 161 pages, is illustrated with eleven plates. The drawings are neatly done and well reproduced. The appearance is marred, however, by the fact that the plates do not fit the page. The work represents the result of years of study on the Pyrenomycetes of North America.—FRED J. SEAVER.

Respecting descriptions in Latin.—Under this heading Dr. Dearness, in the July–August number of Mycologia, criticizes the rule requiring descriptions of new groups to be in Latin or accompanied by a Latin diagnosis. His objections are: that English is almost universally understood; that the Latin description should contain everything that is in the vernacular description and hence the practice is wasteful of space; that the Latin language does not contain words adequate to express the concepts involved.

[1] Smith, Clayton O. *Lepiota Morgani* in Southern California. Mycologia **28**: 86. 1936.

It may be granted that English is intelligible to practically all interested in these matters; so are German and French and, to a somewhat less degree, Spanish, Portuguese and Italian. But what about Finnish, Czech, Polish and Russian, to say nothing of Japanese, Chinese and Hindustani? Surely, the writing of a brief Latin diagnosis is a small price to pay for relief from the necessity of recognizing new groups in all of the many vernaculars in which descriptions might be written were this rule to be generally disregarded. With the strong nationalistic feelings prevalent in the world just now, and likely to be more intensive, rather than less, in the immediate future, agreement on one, two or three of the great modern tongues is not possible with anything like the necessary approach to unanimity.

Need the Latin diagnosis contain everything in the vernacular description? The common practice, at present, is to give the essentials in Latin, and to elaborate upon these in the vernacular. The Latin diagnosis is enough for many purposes, and a very great help in the interpretation of the vernacular discussion when it is necessary that that be consulted. If this seems wasteful of space, an alternative, already used by some competent taxonomists, is available: a full and complete description may be written in Latin and the vernacular discussion omitted.

The argument that Latin words do not exist to express the concepts concerned is completely answered by the very existence of such works as the Sylloge Fungorum. Of course, they do not exist in classical Latin, but new concepts, and the Latin words to embody them, have been introduced into Latin from the days of the Church Fathers until the present. After all, Linnaeus, Fries, Montagne and, above all, the Tulasnes were able to express their ideas in Latin with as great precision and clarity as any of their contemporaries who wrote in their own languages. New concepts and new words in which to express them are still being introduced into taxonomic Latin. We may expect the descriptions of Dr. Dearness' new species to appear in accurate and wholly intelligible Latin, but, unfortunately, only after some years have elapsed. Dr. Dearness is quite right in supposing that this is of small moment when the original descriptions are in English, but it might be much

more serious as a source of future confusion were they in certain other languages.

It may be that the Latin requirement should be abandoned. If so, action to that end should be taken at a representative international congress, after full discussion and consideration. It would seem most unfortunate at a time like this, when the political structure of society is in such anarchy, for scientists to fail, even in so apparently trivial a way, to uphold the ideal of international coöperation, which has been a source of strength in the past and the promise of a better future.—G. W. MARTIN.

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VAN VLOTEN, DR. IR. HEINRICH, Phytopathologist, *Instituut voor Phytopathologie, Laboratorium voor Mycologie en Aardappelonderzoek, Wageningen, Holland.*

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CONSTITUTION AND BY-LAWS

CONSTITUTION

Art. 1. Name. The Society shall be known as the Mycological Society of America.

Art. 2. Membership.

(1) The Society shall consist of members and may include life members, patrons, honorary members, and corresponding members.

(2) Charter membership in the Society shall consist of the persons who, after the invitation of the Secretary, joined before or during the formal organization of the Society at the Atlantic City meetings in 1932.

Art. 3. Dues. The dues for regular members shall be five dollars a year. Any member may become a life member by paying one hundred dollars in one payment, or a patron by paying one thousand dollars, and upon election shall have all the privileges of members. Such funds obtained from life members and patrons shall constitute an endowment fund to be used as may be decided by the Council for the support of mycological publications or projects.

Annual dues of five dollars shall include subscription to the official organ of the Society, and shall be payable in advance on or before December 20. Bills for dues shall be sent to the members in October and it will be necessary to discontinue sending the journal to those whose dues have not been paid by December 20.

Art. 4. Membership and Election of Members.

(1) All persons interested in the study of the fungi shall be eligible to membership.

(2) Members may be elected at any regular meeting of the Society or in the interim between meetings may be elected by the Council. Application for membership must be endorsed by at least one member of the Society.

Art. 5. Officers. The officers of the Society shall consist of a President, Vice-President, and Secretary-Treasurer, whose duties shall be those usually performed by such officers. The President and Vice-President shall serve for one year and the Secretary-Treasurer for three years (or until their successors are elected). Any vacancies occurring in the interim between elections shall be filled by the Council.

The Council shall consist of the President, Vice-President, Secretary-Treasurer, and two Councilors. The Councilors shall be elected, one each year, to serve a term of two years. An individual may not hold two or more positions on the Council at one time.

The Council shall name a Historian to serve for an indeterminate period of years. It shall be the duty of the Historian to accumulate and preserve facts, papers, photographs, and other materials pertinent to a permanent historical record of the Society. The Historian shall not become a member of the Council by virtue of his office as Historian.

Art. 6. Editors and Committees. The editors of the official journal of the Society shall be elected by the Council. The President shall appoint all temporary committees that are to serve during his administration and shall fill all vacancies on standing committees that may occur during his term of office.

Art. 7. Election of Officers. The Secretary-Treasurer shall send to each member of the Society in October a ballot for the nomination of officers. If any nominations are lacking, the Council shall have power to make them. The three candidates for each office receiving the highest number of nominating votes shall be placed upon a final ballot to be sent to ecah member December 1. Should the nominating votes received by a candidate place him among the highest three for more than one office, his name shall appear on the final ballot for only the highest office. The offices rank in the order given in article 5. Votes shall be mailed to the Secretary-Treasurer and counted by the Council. A plurality vote shall elect.

Art. 8. Meetings. An annual meeting shall be held at such time and place each year as the Council may select (usually in connection with the A. A. A. S. meetings). An additional meeting for informal discussion and the carrying out of collecting forays shall be held in the summer or autumn at a time to be selected by the Council. Additional meetings, including special or local meetings for the presentation of papers or the carrying out of forays, may be arranged by the Council at its discretion.

Art. 9. Divisions. Branch organizations or units within the Society known as Divisions, may be established on a geographical basis provided formal application, setting forth the reasons for the establishment of the Division, is made to the parent Society and approved by it.

Art. 10. Journal. The Society shall adopt or establish a journal which shall serve the Society as its official organ primarily for the publication of mycological papers by its members, for the publication of abstracts of the papers delivered at the annual or other meetings, and for the publication of the report of the Auditing Committee or of other reports, announcements, and business of the Society.

Art. 11. Amendments. These articles may be amended by a majority vote of the members voting at any regular meeting of the Society, provided that suggested amendments have been brought to the attention of the Council of the Society in time to be sent to all of the members at least one month previous to the meeting.

BY-LAWS

1. Programs. Programs for annual or other meetings shall be arranged by the Council.

2. Papers. Members wishing to present papers at the annual meeting shall submit to the Secretary-Treasurer the substance and conclusions of the papers in a clear and concise abstract of not more than 200 words. These shall be due on or before November 15, and the Secretary-Treasurer shall be authorized to refuse any received after that date. These abstracts will be edited by the editorial committee of the official journal of the Society for subsequent publication in that organ. Members are urged not to submit titles or abstracts unless they expect to attend the meetings. Except by invitation no member shall offer more than two papers at any one meeting, papers of joint authorship being attributed to the author reading the paper.

3. Associates. Students and others not yet members of the Society may attend meetings and forays in the status of Associates, provided they are recommended to the Council by a member of the Society and pay a fee of one dollar. Such Associates, as they are not members, shall not have the privilege of voting and shall not receive the official journal of the Society, but shall enjoy the other privileges of the meetings and forays including the right to present one paper on the program.

4. Auditing. At each annual meeting the active President shall appoint an auditing committee to audit the accounts of the Society and of its official publication. An audited statement shall be published in the official organ of the Society.

5. Use of the Society's name. Unauthorized use of the name of the Mycological Society of America for advertising or other business ventures is prohibited. The circulation of any unauthorized literature shall be taken as prima facie evidence of the violation of the intent and purpose expressed in this by-law, and the member, after being properly notified, may be expelled from the Society by a majority vote of either the Society at its meetings, or by a majority vote of the Council.

6. These rules may be amended by a majority vote of the members voting at any regular meeting of the Society, provided that suggested amendments have been brought to the attention of the Council of the Society in time to be sent to all the members at least one month previous to the meeting.

CONTRACT WITH THE NEW YORK BOTANICAL GARDEN

The Mycological Society of America hereby adopts Mycologia as its official organ on the following terms:

1. Mycologia will continue to be published by the New York Botanical Garden, the editorial policies to be determined by an Editorial Board, consisting of a Managing Editor appointed by the New York Botanical Garden, and five Editors elected by the Mycological Society of America. The term of office of the five elected editors will be five years, except that at the start they will be designated to serve one to five years respectively. One editor will be elected annually, thereafter, to fill the place of each retiring editor.

The six members of the Editorial Board will elect an Editor-in-Chief from among their number. He will be eligible for repeated reëlection. Final decision of all questions on editorial policy will be made by him, except that the Managing Editor will have full authority in all matters pertaining to the finances of the journal.

2. All personal subscribers now receiving Mycologia may become members of the Mycological Society of America if they so desire. Institutional subscribers to Mycologia are not to be regarded as members of the Society.

3. All members of the Mycological Society of America in good standing will receive Mycologia. In return the Society will transmit to the New York Botanical Garden, through the Managing Editor, four dollars per year for each such member.

4. The New York Botanical Garden agrees to spend on the publication and distribution of Mycologia all funds received from subscriptions, as well as all funds transmitted by the Mycological Society of America. The Garden

further agrees to use for these purposes all sums received from the sale of those volumes of the journal which shall be published after this contract is put in force. Earlier volumes remain the property of the New York Botanical Garden. It is understood that the journal will be used by the Garden for exchange purposes as formerly. Should the contract be terminated, it is agreed by the Mycological Society of America that all excess stock of Mycologia then on hand will be regarded as the property of the New York Botanical Garden.

5. The New York Botanical Garden reserves the fourth cover page to be used without charge for the advertisement of its publications, including Mycologia. The other three cover pages will be used by the Mycological Society of America as it may see fit. All sums collected from paid advertising will be expended on the journal.

6. This contract may be altered at any time by mutual agreement of the New York Botanical Garden and the Mycological Society of America. It may be terminated at the end of any calendar year on six months written notice should it prove unsatisfactory to either party concerned.

7. The contract goes into effect at the beginning of the calendar year 1933.

PAST AND PRESENT OFFICERS OF THE SOCIETY

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1932	Wm. H. Weston, Jr.	1933	G. W. Martin
1933	C. L. Shear	1934	B. O. Dodge
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1936	H. M. Fitzpatrick	1937	L. O. Overholts
1937	John Dearness	1938	E. B. Mains
1938	L. O. Overholts	1939	D. H. Linder
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1940	D. H. Linder	1941	W. H. Snell
1941	E. A. Bessey		

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1939–41	J. N. Couch	1933–34	C. R. Orton
		1934–35	L. O. Overholts
		1935–36	C. L. Shear
		1936–37	B. O. Dodge
		1937–38	H. M. Fitzpatrick
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		1940–41	H. H. Whetzel
		1941–42	F. D. Kern

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1933–34	J. A. Stevenson	1937–41	J. N. Couch (resigned Dec. 1939)
1933–35	F. A. Wolf		
1933–36	G. R. Bisby	1940–41	F. K. Sparrow
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FINANCIAL STATEMENT

January 1, 1939–December 21, 1939

Balance on hand		
Checking account		$ 697.70
Bonds		200.00
Collected in dues		2233.31
		$3131.01
Expenditures		
New York Botanical Garden for Mycologia	$1560.00	
Clerical help	14.50	
Postage	53.00	
Printing and Stationery	71.78	
Yearbook, 1939	76.40	
Secretary's travelling expenses to Richmond	42.75	
Checks returned (later collected)	25.00	
Collection charges	1.16	
	$1844.59	
Balance on hand		
Checking account	$ 586.42	
Savings account	500.00	
Bonds	200.00	
	$1286.42	1286.42
		$3131.01

(signed) J. N. COUCH, *Secretary-Treasurer*

Examined and found correct:

(signed) FRED J. SEAVER, *Chairman, Auditing Committee*

December 21, 1939 to January 1, 1941

Balance on hand	
Checking account	$ 586.42
Savings account	500.00
Bonds	200.00
Collected in dues	1804.30
	$3090.72

Expenditures

New York Botanical Garden for Mycologia	$1492.00
Returned checks (later collected)	20.00
Collection charges	2.68
Central bureau subsidy	25.00
Secretary's supplies (printing, etc.)	43.58
Mycological Foray expense	5.25
Biologists' Smoker	10.00
Secretary's travelling expenses, Chapel Hill, N. C. to Columbus, Ohio	57.24
State tax	.59
	$1656.34

Balance on hand

Checking account	$ 734.38	
Savings account	500.00	
Bonds	200.00	
	$1434.38	1434.38
		$3090.72

(signed) J. N. COUCH, *Secretary-Treasurer*

Examined and found correct:

(signed) FRED J. SEAVER, *Chairman, Auditing Committee*

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